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Effects of ultra high hydrostatic pressure on the structure and properties of starches

Herman Katopo
Iowa State University

Effects of ultra high hydrostatic pressure on the structure and properties of starches

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Herman Katopo

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Herman Katopo

Has met the thesis requirements of Iowa State University

_____
Committee Member

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Committee Member

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Committee Member

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Major Professor

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For the Major Program

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For the Graduate College

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INTRODUCTION

Starch is the major polysaccharide storage in higher plants. It is produced by photosynthesis in amyloplast. Starch can be found in leaves, stems, seeds, fruits, and roots (Fitt and Snyder 1984, Lineback 1984, Swinkels 1985). Most of the starches used are from cereal grains such as maize, wheat, and rice; roots such as tapioca; tubers such as potato; and from inside the bark such as sago. The two major components of starch are amylose and amylopectin. Amylose is essentially a linear macromolecule of glucose units with α-1,4 linkages for the main chain and about 0.5% α-1,6 linkages for the branches. Amylopectin is a branched molecule made from D-glucopyranosyl units with 4%-6% α-1,6 linkages and short linear chains of α-1,4 linkages. Most starches contain 70%-80% amylopectin and 20%-30% amylose (Banks et al. 1974, Greenwood 1979).

Starches are used in different varieties of industries, especially food industries. During processing, starches experience several treatments that cause changes in physical properties. The final product characteristics will be affected by those changes. Most processing involves temperature treatments. Since pressure treatment were known to have similar effects with heat treatment, many scientists looked for the possibility of using high hydrostatic pressure as a way to process starch-based food products. The high hydrostatic pressure technology was around for decades but not until 1990 that the first high pressure processed jam was marketed in Japan by Meida-Ya Food Company (Kimura 1992). Until now, only a few studies have been done on the effects of high hydrostatic pressure on starches. The objective of this study was to investigate the effect of ultra high hydrostatic pressure treatment on the physical and structural properties of starches.

A. Thesis Organization

This thesis consists of a literature review, a paper, "Effects of ultra high hydrostatic pressure on the structure and properties of starches," and general conclusions. The paper contains an abstract, introduction, materials and methods, results and discussions,

conclusions, and references. References cited in the General Introduction and Literature Review follow the General conclusions

LITERATURE REVIEW

A. General Properties of Starch

Starch is a form of energy storage in higher kingdom of plants. It is produced by photosynthesis in the amyloplast. The storage sites can be found in leaves, stems, seeds, fruits, and roots (Fitt and Snyder 1984, Lineback 1984, Swinkels 1985). The size and shape of a starch granule varies with its botanical origin, maturity, and growing condition (Galachowski 1985, Lineback 1984, Morrison and Gadan 1987). Potato starch granules are large oval-shaped with 15-80 μm diameter. Corn starch granules are round or polygonal with 5-20 μm diameter. Tapioca starch granules are round with 3-18 μm diameter and rice starch granules are polygonal with 3-8 μm diameter. There are two major forms of polysaccharides in starch: amylose and amylopectin. Lipids, proteins, and phosphorous are the minor components that also present in starches.

1. Amylose

Amylose is primarily a linear polymer made up from glucose units with α-1,4 glucosidic linkages. Enzymatic studies indicated that the amylose molecule has a small degree of branching by α-1,6 linkages (French 1975, Hizukuri et al. 1981, Takeda and Hizukuri 1986). Each polymer has one reducing end and one non-reducing end. The degree of polymerization (DP), number of glucose residues per reducing end group, of amylose varies with different starch varieties. The range is from 200 to 20,000 DP (Takeda 1987). In general cereal amyloses are smaller than amyloses from tubers or roots.

Amylose forms a random coil configuration in a neutral aqueous salt solution. In the presence of complexing agent, amylose will form a helix with 6-8 glucose units per turn (Banks et al. 1971, Biliaderis and Galloway 1989, Davies et al. 1980, Rundle and French 1943). The interior of the helix contains mostly hydrogen atoms that gives the capability of complexing with molecules that fit within the lumen of the helix (Whistler and BeMiller 1997). Amylose-iodine complex gives a characteristic blue color that can be used for qualitative and quantitative analysis of amylose (Bates et al. 1943, Lansky et al. 1949,

Pfannemuller 1978). Although the amylose single helices has been found to winds in a right-handed spiral (the most stable conformation), a left handed double helices that resist chemical and enzymatic degradation has also been found (Zobel 1992).

2. Amylopectin

Amylopectin is the branched component of starch. It consists of glucose units connected by α-1,4 linkages with 4-6% of α-1,6 linkages at the branch points (French 1973, Greenwood 1964). The average branched-chain length is 20 to 25 glucose units (Manners 1985). However, the average branched-chain length of high-amylose maize amylopectin is above 30 (Jane and Chen 1992, Hizukuri 1985, Hizukuri et al. 1983). The degree of polymerization (DP) of amylopectin is in the range of 10^4-10^5 glucose residues (Hizukuri et al. 1983, Manners 1985, Zobel 1984).

There are three types of amylopectin chains: A, B, and C (Peat et al. 1956). The A-chains are linked to the molecule only by reducing end-group and do not have any other chains attached. The B-chains in addition to being linked as an A-chain, also carry one or more A-chains or other B-chains. The C-chain is the one that posses a free reducing end-group. The ratio of A-chains to B-chains, the degree of multiple branching, can be used to characterize the structure of amylopectin (Atwell et al. 1980, Manners 1985).

Many different types of amylopectin molecular structure models have been proposed and among those the most accepted one is the cluster model (French 1972). The cluster structure can explain the high viscosity and the acid resistance properties of amylopectin. Since the introduction of the cluster model, somewhat similar cluster models that were based on the chain length distribution of amylopectin were reported (Hizukuri 1986, Manners and Matheson 1981, Robin et al. 1974).

Amylopectin only gives a weak violet-reddish-brown color when reacts with iodine due to short branch-chain length. Amylopectin binds 0-1.2 g iodine/100 g while amylose will bind 19.5-20.5 g iodine/100 g.

3. Starch Granule

The ratio of amylose to amylopectin gives the characteristics of starch granule. Most starches contain 70%-80% amylopectin. Nevertheless, some waxy varieties can have almost

100% amylopectin whereas high amylose varieties will only have 30% or less amylopectin (Banks et al. 1974, Greenwood 1979, Kennedy et al. 1987, Young 1984). There also have been several reports on the presence of intermediate fraction which posses different properties from amylose and amylopectin (Greenwood 1979, Lansky et al. 1949, Paet et al. 1952, Takeda and Preiss 1993, Wang et al. 1993, Whistler 1964, Wolff et al. 1955).

The starch granule composes of alternating crystalline and amorphous lamella. The crystalline structure consists of radial arrangement of amylopectin clusters that line perpendicular to the growth rings from hilum to the surface of the granule (French 1984, Whistler and Daniel 1984). Each amylopectin cluster consists of a region with high branching points, the amorphous lamella, and a region where short chains of amylopectin form double helices, the crystalline lamella (French 1972, Jane et al. 1991, Nikuni 1978). Using cross-linking reactions, it was shown that the amyloses were not present in bundles rather than they intersperse among amylopectins (Jane et al. 1992, Jane et al. 1993, Kasemsuwan and Jane 1994). Amyloses in starch granule were also found to be more concentrated at the periphery of granule (Jane and Shen 1993).

The X-ray diffraction method can provide the information about the structure of the crystalline and the relative amount of crystalline to amorphous in the starch granule. There are three different X-ray diffraction patterns for starches: A, B, and C (Sarko and Wu 1978, Zobel et al. 1988). Each type has its own peak characteristic. A-type has a split peak at 17.2° and a single peak at 22°-23° reflection-angle (2θ). The B-type has a single peak at 5.5° and 17.2°, and a split peak at 22°-24° two theta. The C-type is considered to be a mixture of the A and B-type. The difference in the double helices packing causes the different peak patterns. The A-type starches are packed in close orthozombic arrangement while B-type starches are packed in hexagonal arrangement (Sarko and Wu 1978, Zobel et al. 1988).

The B-type starch can be converted into A-type starch by heat and moisture treatment. On the other hand, the transformation from A-type to B-type starch can not be done unless the A-type structure is destroyed and followed by recrystallization of B-type structure (Zobel et al. 1988). The retrograded amylose also gives B-type X-ray pattern. It was found that the amylopectin branch chain-length was responsible for the different type of X-ray pattern (Hizukuri et al. 1983, Hizukuri 1985, Wild and Blanshard 1986). The

amylopectin branch chain-length for A-type starches are short while the B-type's are long. The C-type starches have an intermediate amylopectin branch chain-length.

Besides A, B, and C-type X-ray diffraction pattern, there is also V-type crystalline pattern. V-pattern appears when amylose is in complex with fatty acid, phospholipid, aliphatic alcohol, surfactant or iodine. Nevertheless, the V-pattern can not be observed on starches with less than 30% amylose (Zobel 1988). The V-pattern has peaks at 8°, 13°, and 21° reflection-angle (2θ).

B. Physical Properties of Starch

1. Gelatinization

Gelatinization happens when starch granules are heated in the presence of water, which result in disruption of molecular order (Guilbot and Mercier 1985). This process occurs due to the breaking of inter- and intra-hydrogen bonds and hydrophobic interaction within the starch granule as a result of increasing temperature (Hari et al. 1989). The broken hydrogen bonds are then replaced with water molecules that cause extensive hydration. The presence of water molecules as plasticizer allows the starch molecules to move freely and eventually destroy the native organization of the starch granule. Starch gelatinization can be observed from irreversible granule swelling, loss of birefringence, and loss of crystallinity (French 1984, Guilbot and Mercier 1985).

Different starches will have different gelatinization temperatures. The gelatinization temperature is 62-72°C for normal maize, 63-72°C for waxy maize, 66-120°C for high amylose maize, 58-65°C for potato, 52-65°C for tapioca, and 68-78°C for rice starch (Whistler and BeMiller 1996). Besides the botanical origin, studies have shown that different factors also affect the gelatinization temperature. The factors are the size of starch granules (Banks and Greenwood 1975), the amylose and amylopectin content (Inouchi 1983), the degree of starch crystallinity (Zobel 1984), and the presence of other components (Jane et al. 1996). The presence of sugar will increase gelatinization temperature of starch (Osman 1978) while iodine will lower gelatinization temperature (Sterling 1978). The organic solvents such as liquid ammonia, formamide, formic acid, chloroacetic, and DMSO

can also disrupt the hydrogen bonding within the starch granule or form soluble complexes with starch (Oosten et al. 1984).

2. Pasting

Pasting is a condition that is characterized by swelling of starch granules, leaching of amylose, and eventually disruption of the granules (Atwell et al. 1988). The starch granules when heated in the presence of water will swell and cause the viscosity to increase. The viscosity will keep increasing until it reaches the maximum and with further shearing the viscosity breakdown will occur (such as in Brabender Visco Amylograph and Rapid Visco Analyzer). The breaking of highly swollen granules, which are very fragile, causes the breakdown in viscosity. As the paste cools down, a gel formation from amylose and amylopectin will increase the viscosity again (Zobel 1984). The amylose will reassociate at a much faster rate than amylopectin; therefore the set back viscosity for waxy type starch will be lower.

The amount other substances will also determine the pasting properties of starch. The phosphate groups, such as in potato, will produce a high peak viscosity with a lower set back and clear paste. The lipids, such as in maize, will contribute to more restricted swelling and shear thinning, and more opaque paste (Zobel 1984). The presence of sucrose will increase paste clarity due to the increase in refractive index and the competition for hydrogen bonding with starch. The sodium chloride will reduce paste clarity due to the reduction of ion repulsion (Craig 1989).

3. Retrogradation

Retrogradation is a process occurring when the gelatinized starch paste is aged. The molecules will become more in order and reassociate to develop crystalline structures (Atwell et al. 1988, French 1975). There are some notable characteristics when retrogradation happen: formation of crystallites that resist enzymatic hydrolysis, decrease in light transmission, and loss of the ability to form a blue complex with iodine (Collison 1968). Initial nucleation, propagation or crystal growth, and crystal perfection are the three steps of retrogradation. Since each step optimize at different temperature range, the storage

temperature is an important parameter in affecting the degree of retrogradation. Nucleation increases with decreasing temperature while propagation increases with increasing temperature (Eliasson 1996).

Other factors that affect the rate of retrogradation are starch varieties, starch concentration, pH, and other ingredients such as salt and surfactants (Whistle and BeMiller 1996). Amylose will retrograde at a much faster rate than amylopectin since the mostly linear molecules can associate easily. The retrogradation of amylopectin involves primarily association of the outer linear branches. The maximum amylose retrogradation happens when amylose DP is around 100 (Gidley and Bulpin 1989). The presence of complexing agents and lipids will decrease the extent of retrogradation.

C. Ultra High Hydrostatic Pressure

Application of high hydrostatic pressure (HHP) on food products can be traced back to the work of Hite (1899) who reported a significant reduction in bacteria count in milk after 667 MPa pressure was applied for 10 minutes. In 1914 Bridgman discovered that high pressure coagulated egg albumin. Since then many scientists examined the relationship between HHP and food such as: the effect of pressure on microorganism in raw milk (Thimson and Short 1965), combination of high-pressure and pasteurization temperature on low acid foods (Wilson 1974), effect of high pressure on beef protein quality (Elgasim and Kencick 1980), improvement of refrigerated storage for high pressure treated food (Charm et al. 1977), and reduction of yeast in various juices due to high hydrostatic pressure treatment (Hoover et al.1989).

Those studies led to formation of the Japanese Research and Development Association for High Pressure Technology in Food Industry in 1989. This organization together with food industries, machine industries, and ministry of agriculture, forests and fisheries accelerated the implementation of high-pressure technology in food industry (Hayashi 1992). In 1990, the first high-pressure processed jam was marketed by Meida-Ya Food Company, who further extended their products with variety of fruit yogurts, fruit jellies,

salad dressings, and fruit sauces. Those products retained the vitamin contents, colors, and flavors of fresh fruits and had a longer shelf life (Kimura 1992).

1. Principles of High Hydrostatic Pressure

The HHP will favor a reaction that results in a volume decrease and will retard a reaction with an increase in volume (Hoover et al. 1989). Some example of reactions that are associated with a decrease in volume and enhanced by HHP are the breaking of ionic bonds, formation of hydrogen bonds, and formation of hydrophobic interactions at pressures over 1000 Bar (Suzuki and Tanigachi 1972).

HHP will break the secondary and tertiary structures and leave the covalent bonds intact (Cheftel 1992). Large molecules such as proteins, enzymes, polysaccharides, ribosomes, and cell membranes will be disrupted by HHP while the small molecule like amino acid, vitamin, flavor, fragrance component, and pigment will remain unaffected. Although covalent bonds are not directly affected by HHP, but since the food is a complex multi component system, the reactions between different components can be accelerated (Thevelein et al. 1981). Pressure in HHP treatment acts immediately and independently regardless of the size and the shape of the products (Stute et al. 1996).

Based on the principles of HHP, the potential application are: sterilization or prolongation of storage time, denaturation of proteins, enzyme inactivation, extraction of organic substances, food processing, low temperature freezing and thawing, and control of chemical reactions or organic synthesis (Bergman and Westerland 1994, Pothakamury et al. 1995, Swientek 1992).

2. High Hydrostatic Pressure on Starch

The investigations that have been done so far on the effect of HHP on starch can be divided into three categories. The first one was when the application of pressure was not high enough to gelatinize starch (Thevelein et al. 1981, Vainionpa et al. 1993). Despite the inadequate pressure to cause gelatinization, they found an upward shift of gelatinization temperature compared to untreated starches.

The second one was when pressure application was performed on almost dry starch (Kudla and Tomasik 1992). The application can be considered as compression of starch granules. Kudla and Tomasik found out that the high pressure damaged the starch matrix and produced cracks on the surface of starch granules.

The third one was ultra high pressure treatment. The pressure condition is defined as ultra high pressure (UHP) when the investigation is carried out with excess water and pressure above 400MPa. The result of UHP depends on the moisture content during the pressure treatment (Hayashi and Hayashida 1989, Stute et al. 1996, Yoshiko et al. 1993). With enough water and pressure (above 800 MPa for potato starch) the starch will gelatinize (Kervinen et al. 1995, Muhr and Blanshard 1982, Muhr et al. 1982). Stute et al. (1996) used UHP on starches and examined them with Differential Scanning Calorimetry. They observed that with increasing pressure the gelatinization enthalpy would decrease and a new peak with a peak temperature of about 50°C appeared. They also found that the pressure-treated starch developed weaker gels and displayed lower enzyme susceptibility. On the other hand, Takahashi et al. (1994) found that pressuring a suspension of 10 or 20 mg/mL normal corn starch in 0.01 M acetate buffer above 400MPa enhanced the bindability and digestibility with $Gluc_1$ and $Gluc_2$ (glucoamylase from *Rhizopus sp.*). UHP changed the A-type crystalline structure to B-type (Stute et al. 1996, Yoshiko et al. 1993). They postulated that the change of crystalline structure could be due to some retrogradation that produced resistant starch. This was in agreement with the finding that higher resistance starch was produced when potato starch was gelatinized in high-pressure autoclave (Escarpa et al. 1996).

EFFECTS OF ULTRA HIGH HYDROSTATIC PRESSURE ON THE STRUCTURE AND PROPERTIES OF STARCHES

A paper to be submitted to *Carbohydrate Research*

Herman Katopo, Jay-lin Jane*

ABSTRACT

The structures and physical properties of ultra high hydrostatic pressurized starches were investigated. Six starches were used: normal maize, waxy maize, high amylose maize (70%), tapioca, potato, and rice. Each starch was pressurized in powder form, in 1:1 (v/w) ethanol:starch suspension, in 1:1 and 2:1 (v/w) water:starch suspension, for 5 minutes and 1 hour dwelling time at 100,000 Psi. Pasting and thermal properties were measured by using a rapid visco analyzer and a differential scanning calorimeter, respectively. The molecular weight distribution was analyzed using gel permeation chromatography with Sepharose CL-2B gel followed by detecting using dual channel autoanalyzer. The crystalline pattern was studied using an X-ray diffractometer. The starch granules were observed using scanning electron microscope and polarized light microscope. Starch, which pressurized in the presence of water, gelatinized and the amount of water controlled the degree of gelatinization. The ultra high hydrostatic pressure did not change the molecular weight distribution of the starch. X-ray diffraction studies showed that A-type diffraction pattern changed to B-type-like pattern by the pressure treatments. The DSC thermograms suggested crystalline structure changed during high pressure treatment since a new peak that resembled the retrogradation peak appeared. This agreed with the X-ray diffraction pattern change. The pressurization of powder starches resulted in higher counts of cracking on the surface of starch granules.

* Corresponding author

INTRODUCTION

Starch is the second most abundant polysaccharide found in nature next to cellulose. It is produced by photosynthesis in amyloplast of higher plants. Starch can be found in leaves, stems, seeds, fruits, and roots (Fitt and Snyder 1984, Lineback 1984, Swinkels 1985). The two major components of starch are amylose and amylopectin. Amylose is essentially a linear macromolecule of glucose units with α-1,4 linkages and has a few α-1,6 linkages for the branches. Amylopectin is branched molecule made from glucose units with 4%-6% α-1,6 linkages and short linear chains of α-1,4 linkages. Most starches contain 70%-80% amylopectin and 20%-30% amylose (Banks et al. 1974, Greenwood 1979).

The starch granule is composed of alternating crystalline and amorphous lamella. The crystalline structure consists of radial arrangement of amylopectin clusters that line perpendicular to the growth rings from hilum to the surface of the granule (French 1984, Whistler and Daniel 1984). Using cross-linking reactions, it was shown that the amyloses were not present in bundles, but rather they intersperse among amylopectins (Jane et al. 1992, Jane et al. 1993, Kasemsuwan and Jane 1994). It was also found that the amyloses in starch granule were more concentrated at the periphery of granule (Jane and Shen 1993).

Application of high hydrostatic pressure (HHP) on food products can be traced back to the work of Hite (1899) who reported a significant reduction in bacteria count in milk after 667 MPa pressure was applied for 10 minutes. In 1914 Bridgman discovered that high pressure coagulated egg albumin. The HHP will favor a reaction that results in a volume decrease and will retard a reaction with an increase in volume (Hoover et al. 1989). HHP will break the secondary and tertiary structures and leaves the covalent bonds intact (Cheftel 1992). Although covalent bonds are not directly affected by HHP, but since the food is a complex multi component system, the reactions between different components can be accelerated (Thevelein et al. 1981). Pressure in HHP treatment will act immediately and independently regardless of the size and the shape of the products (Stute et al. 1996).

The investigations that have done so far on the effect of HHP on starch can be divided into three categories. The first one was when the application of pressure was not high enough to gelatinize starch (Thevelein et al. 1981, Vainionpa et al. 1993). The second one

was when pressure application was performed on almost dry starch (Kudla and Tomasik 1992). The third one was ultra high pressure treatment. The pressure condition is defined as ultra high pressure (UHP) when the investigation is carried out with excess water and pressure above 400MPa. The X-ray studies showed UHP changed the A-type crystalline structure to B-type (Stute et al. 1996, Yoshiko et al. 1993). They postulated that the change of crystalline structure could be due to some retrogradation that produced resistant starch. This is in agreement with the finding for higher resistance starch when potato starch is gelatinized in high-pressure (1000 bar) autoclave (Escarpa et al. 1996).

Since very few studies have been done using UHP, the objective of this study was to provide a better insight for the effect of UHP on the physical and structural properties of starches.

MATERIALS AND METHODS

- *Materials*

Normal, high amylose (70%), and waxy maize were gifts from Cerestar USA (Hammond, IN). Tapioca starch was a gift from National Starch and Chemical Company (Bridgewater, NJ). Potato and rice starches were purchased from Sigma Chemical Co. (St. Louis, MO).

- *High Hydrostatic Pressure Treatments*

The starch samples were pressurized to 100ksi (690 MPa) at room temperature for 5 minutes and 1 hour dwelling time with a warm isostatic high hydrostatic pressure unit (Engineered Pressure System Inc., Andover, MA). The unit was an indirect-compression system using a high-pressure intensifier (Critter P60-03, Hydro-Pac Inc., Fairview, PA) to pump the pressure medium, 5% hydraulic oil in distilled water, from the reservoir into the closed vessel, until the desired pressure was reached. The internal measurements of the pressure vessel were 101.6 mm for the diameter and 279.4 mm for the height.

Each sample was pressurized under four different conditions: powder form (original moisture content: 10 %-16 % dbs), suspended in ethanol at 1:1 (v/w) ratio, suspended in

water at 1:1, and 2:1 (v/w) ratio. One hundred grams of starch were used for each treatment. The sample was double bagged into nylon polyethylene plastic bags (thickness = 3 mm) (Curwood Inc., Chicago, IL) and double sealed using Fresh Vac (CVP System Inc., Downers Grove, IL).

The samples that were pressurized in powder form turned into a solid rock. Therefore, it needed to be broken into smaller pieces by using a hammer and then ground with a cyclone mill (UDY Corp., Fort Collins, CO). The ethanol pressurized samples were still in suspension so they were vacuum filtered and dried in an oven at 40°C for approximately 24 hr. All the starches that were pressurized in the presence of water formed a cake or gel that had to be broken into smaller pieces, and dehydrated using excess ethanol and then vacuum filtered, dried, and ground with a cyclone mill. The moisture content was determined by drying at 110°C oven for 24 hours.

- Polarized Light Microscope

The starch was suspended in a glycerol solution (glycerol:H_2O = 2:1 v/v) to produce 1 % solid suspension. The sample was then observed using Nikon biological microscope that was equipped with polarized filter (Labophot, Tokyo, Japan). The micrograms were taken using Nikon camera (FX-35WA, Tokyo, Japan) attached at the top of the microscope with phase contrast equipment from Nikon (HFX-11, Tokyo, Japan).

- Scanning Electron Microscope

A one percent starch suspension in absolute ethanol was prepared for each sample. One drop of the suspension was dropped on the non-sticky side of aluminum tape that was attached to a brass disc. The specimens were coated in a Polaron E5100 sputter coater with gold to palladium ratio of 60 to 40. The prepared samples were observed using a JEOL JSM-35 scanning electron microscope at 10 kV (Tokyo, Japan).

- X-ray Diffraction

The X-ray patterns of starches were obtained with copper (nickel foil-filtered) K_α radiation using a diffractometer (D-500, Siemens, Madison, WI). The samples were first

equilibrated in a 100% relative humidity chamber for 24 hours at room temperature. The operation setting for diffractometer was 27 mA and 50 kV. The angle of diffraction (2θ) scanned was from 4° to 40° with 0.05° step and 2 seconds count time.

- Differential Scanning Calorimetry

The gelatinization properties of starch were analyzed using Perkin-Elmer Differential Scanning Calorimetry (DSC-7, Norwalk, CT) following the method of Chen and Jane, 1994. The amount of sample used for tapioca, rice, potato, native and waxy maize starch was approximately 2 mg (dbs) each with the addition of 6 μL deionized water. Those samples were sealed in aluminum pans (Perkin-Elmer), equilibrated for one hour and scanned. The heating rate was 10°C per minute over the temperature of 25-110°C. Stainless steel pans were used for high amylose maize starch with approximately 10 mg (dbs) starch and the addition of 30 μL of deionized water with heating until 140°C. The data were averaged from a minimum of three replicates of each starch sample. The total weight of the starch cake or gel was used for the DSC scanning performed right after pressure treatment and the moisture content were determined later by putting the punctured pan into 110°C oven for 24 hours.

- Rapid Visco Amylograph

The pasting profiles were obtained using a Rapid Visco Analyzer (RVA) (Newport Scientific, Sydney, Australia). All the samples were tested at 8% w/w solid concentration (28 g total weight) except for waxy maize samples, which were run at 4% w/w solid concentration. The high amylose maize could not be tested since it required a much higher temperature to gelatinize. The samples were equilibrated at 50°C for 1 minute and then heated at the rate of 6°C/min to 95°C and maintained at that temperature for 5 minutes before cooling to 50°C at the rate of 6°C/min. A constant 160 rpm spindle speed was used. Two replications with two repetitions were done for each sample.

- Gel Permeation Chromatography

The analysis was done by using the methods of Jane and Chen (1992). Starch (0.25g) was suspended in 2.5 mL deionized water and 22.5 mL DMSO. The suspension was boiled

and stirred in boiling water bath for 2 hours and then continuously stirred at room temperature for 24 hours. Three milliliters of suspension (only 1 ml for high amylose maize) was precipitated with about 20 ml of ethanol. The precipitate was separated by centrifugation at 7000 rpm for 15 minutes and then dissolved in 1 mg/10 mL glucose solution and heated in boiling water bath for 20 minutes. Five milliliters of the solution was then injected into a 2.6 x 80 cm column (Pharmacia Inc., Piscataway, NJ) packed with Sepharose CL-2B gel. The eluent used was an aqueous solution with 2.5 mM NaCl and 1 mM NaOH at the flow rate of 0.5 ml/min in ascending direction. Fractions of 4.8 mL were collected and analyzed with a dual channel Autoanalyzer II (Technicon Instrument Corp., Elmsford, NY). The total carbohydrate (determined by anthrone-sulfuric acid method) and the amylose-iodine blue value was measured at 630 nm and 640 nm, respectively. Since the height for the curve fluctuated between runs, normalization of the chromatogram was done. The fraction number was normalized by setting the amylopectin peak to zero and glucose peak to one. The total carbohydrate and blue value was normalized by setting the amylopectin peak height to one.

- Control

The starch samples (normal maize, waxy maize, tapioca, and rice) were prepared the same way as for pressure treatment in water suspension, but for control the starches were gelatinized in boiling water bath. After cooling down the same condition was applied as the direct measurement of gelatinized starch using DSC as described above.

RESULTS AND DISCUSSIONS

Since the results from 5 minutes pressurization were very close with the 1 hour results, the only data presented here were the 5 minutes results.

- Morphology of HHP Treated Starches

All the starches that were pressurized in powder form became a solid hard rock. The ones pressurized in ethanol suspension did not show any apparent change. The starches that

were pressurized in the presence of water became a cake or gel, except for high amylose maize that did not change at all.

The pressurization in 1:1 (v/w) water to starch suspension turned the normal corn starch into a yellowish brittle gel; a softer gel was observed when the water ratio was doubled. The waxy maize turned into a light-brown hard gel upon pressurization in 1:1 (v/w) water to starch suspension. The gel became very sticky when the water ratio was double for the waxy maize. The tapioca starch appeared to be like waxy maize but the color for tapioca gel was brown and the gel was not as sticky as that of waxy maize. The potato starch in 1:1 (v/w) water to starch suspension turned into a brittle cake with a lot of free water. In 2:1 (v/w) water to potato starch suspension, the cake became softer with a lot of free water and a thin layer of gel on the surface of the cake. The appearance for pressurized rice starch was very much like normal maize's but the color was white.

- By Polarized Light Microscope

The light microscope micrographs of normal maize starch are presented in Figure 1-3. The micrographs showed that the starches that were pressurized in powder form and in ethanol suspension did not change their morphology (Fig 2). Although higher amounts of granule surface cracking were noticed for dry powder form pressurization. The starches that were pressurized in 2:1 (v/w) water to starch suspension showed a higher degree of gelatinization compared with pressurization in 1:1 (v/w) water to starch suspension (Fig 3). The gelatinized starch became more transparent like a membrane enclosing the granules that did not gelatinize. The only starch that did not have any appearance change was the high amylose maize. The high amylose maize did not even show any gelatinization. The potato starch showed a lower degree of gelatinization compared with other starches, except high amylose maize.

- By Scanning Electron Microscope

The micrograms of SEM showed that when the starches were pressurized in the presence of water gelatinization occurred and the gelatinized starch dispersed and surrounded

the granules that were not gelatinized. The micrograms of normal and waxy maize starch pressurized in suspension of water (1:1) could be seen in Figure 4.

- X-ray

The X-ray diffraction patterns of native and pressure treated starches in water suspension are shown in Figure 5-10. The results showed that waxy maize starch went through a change in the X-ray pattern from A toward B-like pattern (Fig 6). The pattern became a combination of A and B-type pattern (C-pattern). The appearance of peak at around 5^{o} and the transformation from double peak to single peak at around 17.5^{o} was the characteristic of B-type X-ray diffraction pattern. Nevertheless, the single peak around 22^{o} that is the characteristic of A-type X-ray diffraction pattern did not change into double peak. The X-ray diffraction pattern of pressure treated normal maize starch in water media also showed a strong peak at about 20^{o} and weak signals at 13^{o} and 8.5^{o}, indicating the presence of amylose-lipid complex (V-pattern) (Fig 5).

The X-ray diffraction pattern of normal and waxy maize starch pressurized in powder form and in ethanol suspension are shown in Figure 5 and 6, respectively. There were no change for X-ray diffraction pattern from A-type to B-type. Only the decrease in the intensity of the peaks due to some lost of crystallinity. Therefore, only those representatives are shown. The intensities of X-ray diffraction peaks for pressurization of powder starches were lower than the ones in ethanol suspension. This suggested the starches in ethanol suspension were more stable to HHP treatments.

The change of X-ray pattern for tapioca starch (Fig 7) did not turn out to be as pronounce as for normal and waxy maize. There was a small peak appeared at round 5^{o} and the double peak around 17.5^{o} became single peak. Those indicated the change toward B-type X-ray pattern. The X-ray diffraction pattern for rice starch (Fig 8) also a change toward a weak combination of V- and B-type but the peaks diminished as a result of starch gelatinization. The treated potato and high amylose starch in aqueous media for different length (Fig 9 and 10) kept its original X-ray pattern (B-type).

- *Differential Scanning Calorimetry*

The DSC result was presented in Table 2. The peak one was the peak that resembled the retrogradation peak and the peak two was the gelatinization peak. There was also peak three that was the amylose-lipid complex for native maize, tapioca, and rice starch, but since the magnitude between runs varies a lot so the results were not presented in the table. There were some variations for the results because the starch suspension tended to settle during high pressure treatment. The sample suspension was not homogeneous, and hence the degree of gelatinization could vary depend on water availability.

For normal maize, the pressure treatment in powder state decreased the T_o, T_p, T_c, and ΔH of the starch compared with the native. This indicated some loss of crystallinity resulted from compression. The effect was not significant for the normal starch suspended in ethanol to give change in the onset temperature, but the enthalpy change also decreased hence indicated some loss of crystallinity. The degree of gelatinization for normal maize was higher for 2:1 then 1:1 water to starch ratio since the gelatinization peak could not be detected in 2:1 suspension. There were some differences for peak one results between the directly measured right after pressure treatment and the ones that were analyzed later. This variation could be the result from further retrogradation during storage and waiting before measurement. Also since the peaks were very small, the reading could also vary.

The DSC result for waxy maize had the same trend as that of the normal-maize starch. The dry powder pressurization also had lower results compared with the native and in ethanol suspension. The fact that waxy maize gel was more difficult to dry made the degree of retrogradation higher compared with the ones measured directly right after pressure treatment. The high pressure treatment did not really affect the high amylose maize. Nevertheless, the decreased for the enthalpy change suggested some loss of crystallinity. Since the peaks were broad, the results varied.

The same trend appeared for tapioca and rice starch which were pressurized in powder form and in ethanol suspension as the previously reported starches. The peaks that resembled retrogradation peak were very small and not always detectable. The pressure application for potato starch only resulted in a very limited gelatinization. Even in the excess amounts of water, the gelatinization only occurred on the surface of the cake. Since the

sample was not homogenous, the variation in ΔH was fairy large. The results indicated that starch was most stable in EtOH while under high pressure treatments.

The control experiment resulted in no peak at all. That indicated the peak that resemble retrogradation peak was generated during high pressure treatments and not from retrogradation after pressurization. Since all the starches were completely gelatinized, the second peak also disappeared. Therefore, the DSC results were not presented in table form (no peaks).

- Rapid Visco Amylograph

The RVA result was presented in Figure 11-15. The amylogram for the unpressurized normal maize starch (Fig 11) showed the highest peak viscosity and set back. There was no significant difference between the normal maize pressurized in the powder form and in ethanol suspension. Pressure treatments of normal maize in aqueous media did result in substantial variations in their pasting properties: increasing pasting temperature and decreasing peak viscosity. The differences were more severe for 2:1 (water:starch) than 1:1 (water:starch). These differences could be attributed to the destruction of native crystalline structure that consisted of double helical crystalline structure of amylopectin while amylose is present in the amorphous region. During the pressure treatment, the native A-type crystalline structure was destroyed and B-type crystalline structure developed among amylopectin and amylose. Dispersion of amylose/amylopectin crystallite required more energy, and thus, it showed higher pasting temperature and restricted swelling as low peak viscosity and little shear thinning. The values for RVA analysis results are shown in Table 1.

The reason for choosing 4% solid when analyzing waxy maize was the appearance of a sharp shoulder during the holding of temperature at 95°C if the 8% solid were used. The unpressurized waxy starch had a slightly higher peak viscosity, than those treated in powder form and in ethanol suspension (Fig 12). The treatments with 2:1 water to starch ratio indeed increased the degree of gelatinization that some starch started pasting at 50°C.

Figure 13 showed the RVA curve for tapioca starch. The starch treated in ethanol suspension showed identical profiles with the unpressurized tapioca starch. During pressure treatment, the dry powder tapioca starch suffered more damage (cracking) by compression.

This could be the reason for lower pasting temperature and higher peak viscosity for the dry powder pressurization of tapioca starch. The tapioca starch that was pressurized in the presence of water showed a much higher peak temperature and set back viscosity. This indicated that the partially gelatinized starch did not necessarily need to have a lower peak viscosity and lower set back than the native. The high set back viscosity could be caused by interaction between amylose and amylopectin during the pressure treatment in water media.

Figure 14 showed the RVA curve for rice starch. Like most other starches, the result for dry powder pressurization was not significantly different from the native rice starch. In contrast to potato and tapioca starches, there was a little decrease for the peak viscosity and the pasting temperature for the rice starch pressurized in ethanol suspension. This could be attributed to the removal of lipids by ethanol. A small peak that resembled the one in normal maize starch also appeared in the rice starch for those pressurized in the presence of water.

The result for dry powder, ethanol, and native potato starch seemed to be very close (Figure 15). The pressurization of potato starch in the presence of water increased the set back viscosity and delayed the pasting, but the peak viscosity did not decrease much since only small amount of starch gelatinized (as seen in microscope observation). The possible reason for the delayed of pasting temperature was increasing crystalline structure as indicated by DSC enthalpy change data.

- Gel Permeation Chromatography

The results for GPC are presented in Figure 16-19 as the normalized total carbohydrate or blue value versus normalized fraction number. The total carbohydrate curves for normal maize (Fig 16a), high amylose maize (Fig 19a), and tapioca (Fig 18a) were very close between different treatments except for waxy maize (Fig 17a)that had a slight shift. This indicated no molecular weight break down during the high pressurization treatments. The reason why the amylose peak height for high amylose maize varied could be caused by a slight difference in the amounts of sample injected. Since the total amount of sample used for high amylose maize was much less that the rest of the samples, a small variation could magnify easily. The blue value curves seemed to fluctuate more than the

total carbohydrate curves. This mainly caused by the sensitivity of the instrument (the photo detectors were very old) and a possible slight variation of the iodine strength used for each run. The location of the peaks were pretty much the same that indicated the same molecular weight distribution.

CONCLUSIONS

The high hydrostatic pressure treatments of starch in the presence of water resulted in gelatinization at room temperature. Water played important rule to control gelatinization since the presence of ethanol prevented gelatinization even at maximum pressure tested. When little ungelatinized starch granules were present, the DSC could not detect their presence but polarized light microscope displayed some granules with weak birefringence. The pasting properties of partially gelatinized starches from UHP treatment changed depending on the source of starches. Different starches gave different response to UHP treatment. In general, there was no significant change between the treatments with 5 minutes and 1 hour dwelling time. GPC results showed there was no molecular degradation resulting from high hydrostatic pressure treatment. X-ray diffraction patterns of treated A-type starches were changed towards B-type patterns, and the starches displayed an additional new peak (To between 41-46^{o}C) in DSC thermograms suggesting crystalline structure changes occurred during pressure treatment (from control experiment). The changing from A-type pattern into B-type pattern was in agreement with Yosikho et al. (1993), and Stute et al. (1996). Type B X-ray pattern starches were more resistant to high pressure treatment. The DSC results showed all methods of pressure application would at least destroy some crystallinity by decreasing the enthalpy change. Microscope results revealed that dry powder compression caused more surfaces cracking, hence altering some pasting characteristics compared with the native starch. This finding was in agreement with the dry compression of potato starch at a much higher pressure done by Kudla and Tomasik (1992).

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FIGURE LEGENDS

Figure 1. The micrograph of normal maize: under normal light (top), under polarized light (bottom).

Figure 2. The micrographs of normal maize pressurized in powder form (left) and in 1:1ethanol suspension (v/w) (right) for 5 minutes: under normal light (top), under polarized light (bottom).

Figure 3. The micrographs of normal maize pressurized in 1:1 water suspension (v/w) (left) and 2:1 water suspension (right) for 5 minutes: under normal light (top), under polarized light (bottom).

Figure 4. The micrograms from SEM for normal maize starch (left) and waxy maize starch (right): control (top), pressurized at 100 ksi in 1:1 water:starch (v/w) ratio for 5 min (bottom)

Figure 5. The X-ray diffraction pattern for normal maize starch pressurized at 100 ksi.

Figure 6. The X-ray diffraction pattern for waxy maize starch pressurized at 100 ksi.

Figure 7. The X-ray diffraction pattern for tapioca starch pressurized at 100 ksi.

Figure 8. The X-ray diffraction pattern for rice starch pressurized at 100 ksi.

Figure 9. The X-ray diffraction pattern for potato starch pressurized at 100 ksi.

Figure 10. The X-ray diffraction pattern for high amylose maize starch pressurized at 100 ksi.

Figure 11. The RVA curve for normal maize starch with 8% solid concentration and 160 rpm spindle speed.

Figure 12. The RVA curve for waxy maize starch with 4% solid concentration and 160 rpm spindle speed.

Figure 13. The RVA curve for tapioca starch with 8% solid concentration and 160 rpm spindle speed.

Figure 14. The RVA curve for rice starch with 8% solid concentration and 160 rpm spindle speed.

Figure 15. The RVA curve for potato starch with 8% solid concentration and 160 rpm spindle speed.

The abbreviation for legends in Figure 11-15 are "m" for minutes, "Dry Pwd" for powder form, "EtOH" for suspension in ethanol, "1:1 and 2:1" for the ratio of water to starch suspension, and "Temp" for temperature.

Figure 16a. The total CHO curve from 15 mg of normal maize starch separated in Sepharose CL-2B column.

Figure 16b. The blue value curve from 15 mg of normal maize starch separated in Sepharose CL-2B column.

Figure 17a. The total CHO curve from 15 mg of waxy maize starch separated in Sepharose CL-2B column.

Figure 17b. The blue value curve from 15 mg of waxy maize starch separated in Sepharose CL-2B column.

Figure 18a. The total CHO curve from 15 mg of tapioca starch separated in Sepharose CL-2B column.

Figure 18b. The blue value curve from 15 mg of tapioca starch separated in Sepharose CL-2B column.

Figure 19a. The total CHO curve from 5 mg of 70% amylose maize starch separated in Sepharose CL-2B column.

Figure 19b. The blue value curve from 5 mg of 70% amylose maize starch separated in Sepharose CL-2B column.

The abbreviation for legends in Figure 16-18 are "NCS" for normal maize starch, "WX" for waxy maize starch, "70%" for 70% amylose maize starch, "DP" for Powder form, "Et" for suspension in ethanol, "W" for suspension in water, "m" for minutes, "1:1 and 2:1" for the ratio of water to starch.

Table 1. The RVA result for starches pressurized at 100 ksi for 5 min

Type	Peak (RVU)	Trough (RVU)	Final Visc (RVU)	Pasting Temp (°C)
Normal Maize:				
Native	162.00	101.58	188.75	83.50
in powder form	145.75	92.50	169.50	84.35
in ethanol suspesion (1:1)	135.75	82.83	154.50	84.30
in water suspension (1:1)	65.83	57.25	70.00	89.60
in water suspension (2:1)	40.67	34.58	44.58	94.00
Rice:				
Native	111.67	86.67	137.17	89.55
in powder form	107.08	81.50	126.67	88.30
in ethanol suspesion (1:1)	100.00	69.75	124.08	85.10
in water suspension (1:1)	50.83	37.83	56.58	62.30
in water suspension (2:1)	49.42	36.83	55.50	63.20
Waxy Maize:				
Native	56.00	39.67	46.08	72.35
in powder form	54.92	38.17	46.58	71.15
in ethanol suspesion (1:1)	54.92	37.67	45.67	72.35
in water suspension (1:1)	53.33	31.67	38.42	71.15
in water suspension (2:1)	54.58	29.08	33.67	50.00
Tapioca:				
Native	263.00	88.42	165.50	66.35
in powder form	292.67	92.25	168.00	63.90
in ethanol suspesion (1:1)	258.83	89.92	162.08	65.95
in water suspension (1:1)	282.92	176.83	314.67	66.30
in water suspension (2:1)	231.33	174.67	316.25	57.15
Potato:				
Native	760.58	189.67	256.17	63.55
in powder form	760.25	188.17	247.17	61.55
in ethanol suspesion (1:1)	747.42	169.17	240.42	61.95
in water suspension (1:1)	730.92	267.50	360.08	65.15
in water suspension (2:1)	675.42	313.67	433.17	65.90

Table 2. The Thermal properties of 5 min HHP treated starches determined by Differential Scanning Calorimetry

Starch Type	Peak 1				Peak 2			
	To[a] (°C)	Tp[b] (°C)	Tc[c] (°C)	ΔH[d] (J/g)	To (°C)	Tp (°C)	Tc (°C)	ΔH (J/g)
Normal Maize:								
Untreated	ND	ND	ND	ND	66.0±0.1[sd]	70.1±0.2	80.2±0.0	14.0±0.7
in powder form	ND	ND	ND	ND	61.1±0.1	67.2±0.0	78.7±0.3	11.2±0.7
in EtOH (1:1)	ND	ND	ND	ND	65.8±0.4	69.7±0.4	81.5±1.3	13.1±0.4
in H_2O (1:1)	41.3±0.6	49.4±2.5	57.7±1.1	0.5±0.2	63.3±0.4	69.5±0.5	78.1±0.9	1.9±0.1
in H_2O (1:1), Di	44.8±1.1	51.4±0.1	61.9±0.1	0.9±0.1	65.3±0.7	72.5±0.5	79.9±1.1	1.7±0.3
in H_2O (2:1)	40.8±1.5	50.3±1.8	61.9±0.5	0.6±0.1	ND	ND	ND	ND
in H_2O (2:1), Di	46.2±0.4	51.7±0.7	62.8±0.5	0.3±0.0	ND	ND	ND	ND
Waxy Maize:								
Untreated	ND	ND	ND	ND	64.6±0.2[sd]	70.4±0.3	81.2±0.4	16.1±0.2
in powder form	ND	ND	ND	ND	62.2±0.2	68.4±0.2	80.3±0.8	14.1±0.4
in EtOH (1:1)	ND	ND	ND	ND	64.3±0.2	70.2±0.2	81.9±0.4	15.3±0.1
in H_2O (1:1)	41.8±0.8	47.1±0.6	58.4±1.2	0.7±0.2	64.9±0.1	71.3±0.3	81.3±1.1	3.1±0.4
in H_2O (1:1), Di	43.5±1.7	53.1±1.0	62.9±0.3	1.5±0.3	67.0±0.4	74.1±0.4	84.1±0.7	3.5±0.2
in H_2O (2:1)	40.8±0.5	50.6±0.1	72.3±0.5	4.3±0.5	ND	ND	ND	ND
in H_2O (2:1), Di	40.3±1.6	58.7±2.4	67.6±3.2	0.4±0.1	ND	ND	ND	ND
Tapioca:								
Untreated	ND	ND	ND	ND	64.9±0.1[sd]	69.1±0.1	82.2±0.5	14.8±0.3
in powder form	ND	ND	ND	ND	59.8±0.1	66.2±0.1	78.8±0.2	12.2±0.1
in EtOH (1:1)	ND	ND	ND	ND	64.5±0.1	68.7±0.1	82.0±1.0	14.1±0.2
in H_2O (1:1)	ND	ND	ND	ND	61.5±2.4	68.3±2.4	77.2±2.8	2.2±0.1
in H_2O (1:1), Di	42.5±0.9	48.6±1.9	57.4±0.1	0.2±0.0	63.0±0.3	70.7±0.3	81.6±1.4	2.6±0.1
in H_2O (2:1)	45.6±1.3	47.2±1.4	55.7±1.9	0.2±0.1	ND	ND	ND	ND
in H_2O (2:1), Di	ND	ND	ND	ND	ND	ND	ND	ND

a,b,c,d are onset, peak,and completion temperature, and enthalpy change, respectively.

sd is standard deviation. ND is not detected. Di is directly after pressure treatment.

Table 2. (continued)

Starch Type	Peak 1				Peak 2			
	To[a] (°C)	Tp[b] (°C)	Tc[c] (°C)	ΔH[d] (J/g)	To (°C)	Tp (°C)	Tc (°C)	ΔH (J/g)
Rice:								
Untreated	ND	ND	ND	ND	60.6±0.6[sd]	68.3±0.1	84.7±2.3	16.8±4.1
in powder form	ND	ND	ND	ND	55.7±0.3	65.9±0.3	82.9±0.4	14.1±0.5
in EtOH (1:1)	ND	ND	ND	ND	60.1±0.3	68.1±0.1	84.8±3.2	16.6±2.3
in H_2O (1:1)	ND	ND	ND	ND	64.3±0.4	70.3±0.4	80.4±0.5	0.5±0.1
in H_2O (1:1), Di	46.6±0.2	51.8±0.4	58.2±0.7	0.1±0.0	65.6±0.7	75.2±1.5	84.1±1.7	2.3±0.1
in H_2O (2:1)	44.3±0.0	50.5±0.0	56.6±0.0	0.1±0.0	ND	ND	ND	ND
in H_2O (2:1), Di	44.2±0.0	50.8±0.0	57.0±0.0	0.4±0.0	ND	ND	ND	ND
Potato:								
Untreated	ND	ND	ND	ND	58.1±0.1[sd]	62.4±0.1	72.5±0.1	18.9±0.3
in powder form	ND	ND	ND	ND	54.4±0.8	60.8±0.3	71.4±0.4	17.3±0.4
in EtOH (1:1)	ND	ND	ND	ND	57.1±0.3	61.5±0.3	71.3±0.6	18.4±0.2
in H_2O (1:1)	ND	ND	ND	ND	57.7±0.2	62.3±0.3	71.6±0.5	14.5±0.5
in H_2O (1:1), Di	ND	ND	ND	ND	58.0±0.1	63.3±0.1	76.0±1.7	13.3±0.2
in H_2O (2:1)	ND	ND	ND	ND	56.0±0.2	62.5±0.2	71.9±0.5	9.9±0.3
in H_2O (2:1), Di	ND	ND	ND	ND	58.3±0.5	64.1±0.9	74.5±0.7	12.5±9.5
70% amylose maize:								
Untreated	ND	ND	ND	ND	70.6±0.3[sd]	85.7±0.7	113.1±0.9	13.0±1.0
in powder form	ND	ND	ND	ND	70.4±1.1	96.7±6.8	116.3±3.5	10.6±1.0
in EtOH (1:1)	ND	ND	ND	ND	69.7±0.5	92.1±7.0	112.8±0.7	12.8±0.5
in H_2O (1:1)	ND	ND	ND	ND	72.6±0.5	90.0±7.2	112.7±1.3	8.7±0.7
in H_2O (2:1)	ND	ND	ND	ND	76.5±1.7	100.1±1.4	113.6±1.2	9.5±0.4

a,b,c,d are onset, peak,and completion temperature, and enthalpy change, respectively.

sd is standard deviation. ND is not detected. Di is directly after pressure treatment.

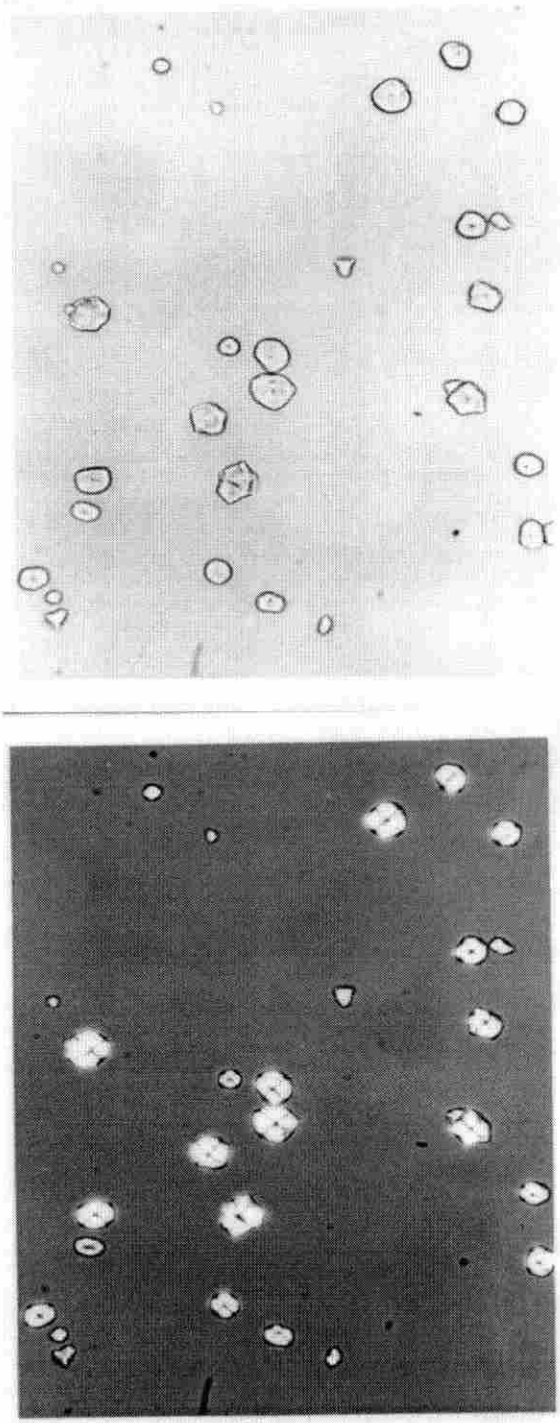

Figure 1.

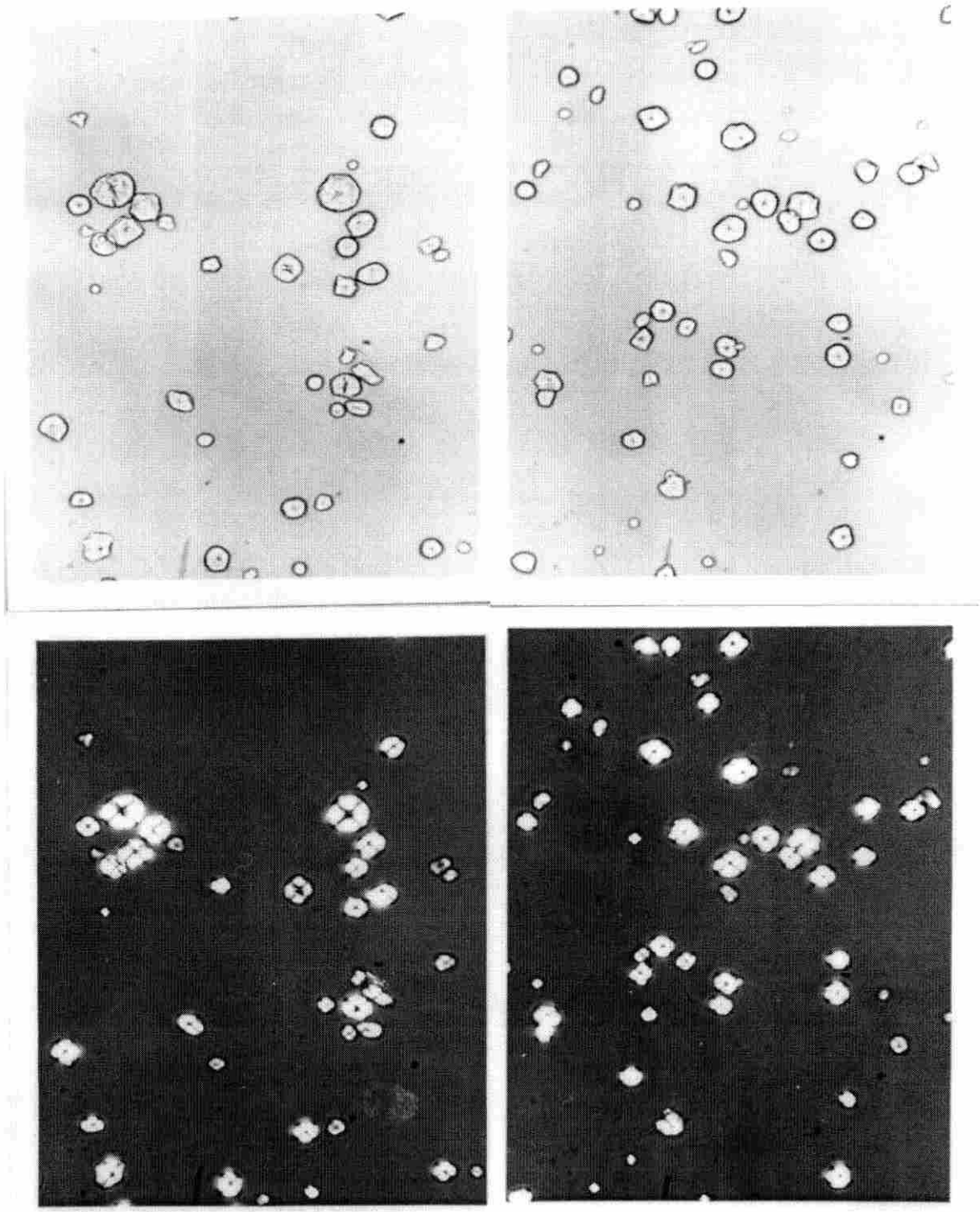

Figure 2.

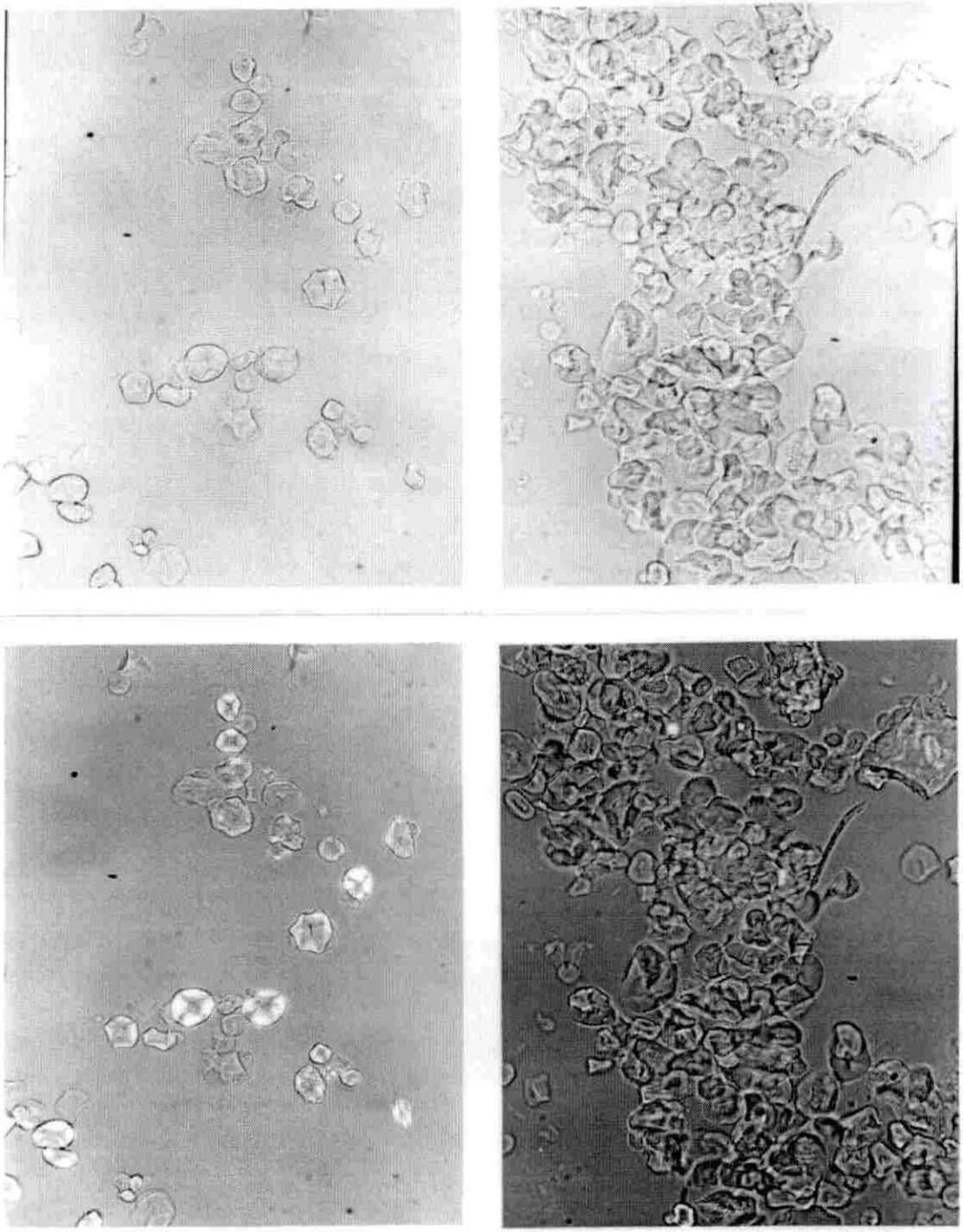

Figure 3.

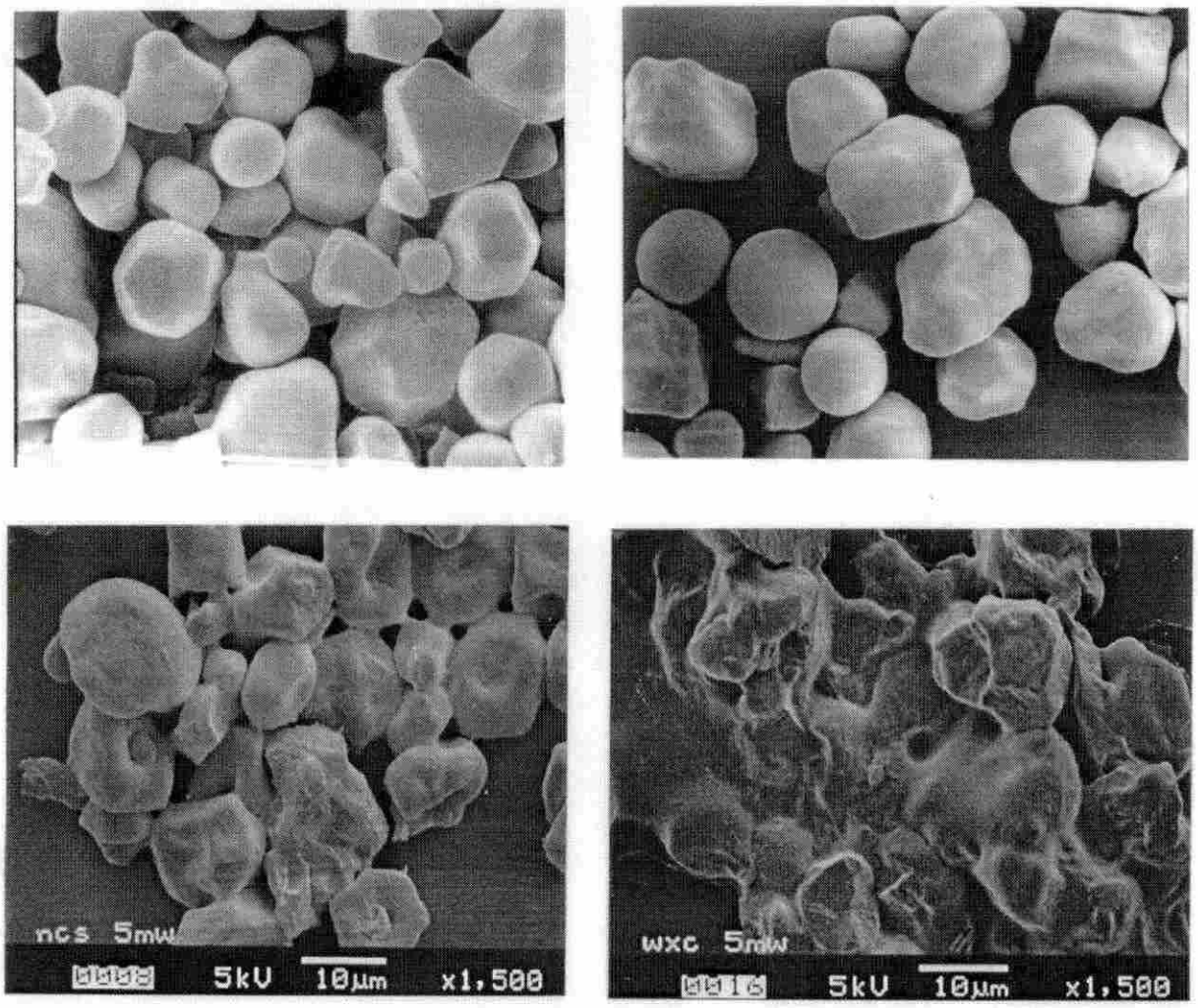

Figure 4.

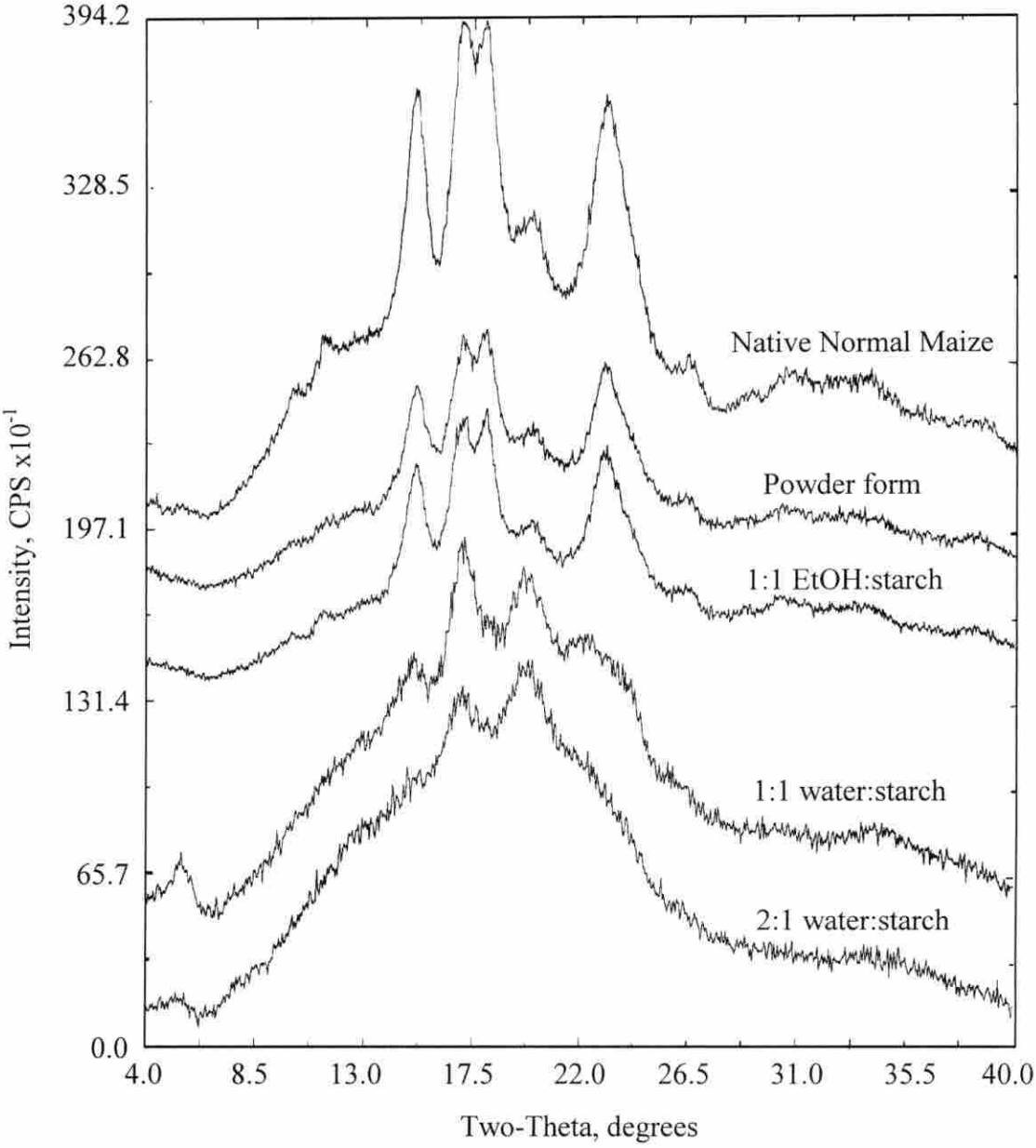
394.2
328.5
262.8
197.1
131.4
65.7
0.0
Intensity, CPS x10-1
4.0
8.5
13.0
17.5
22.0
26.5
31.0
35.5
40.0
Two-Theta, degrees
Native Normal Maize
Powder form
1:1 EtOH:starch
1:1 water:starch
2:1 water:starch

Figure 5.

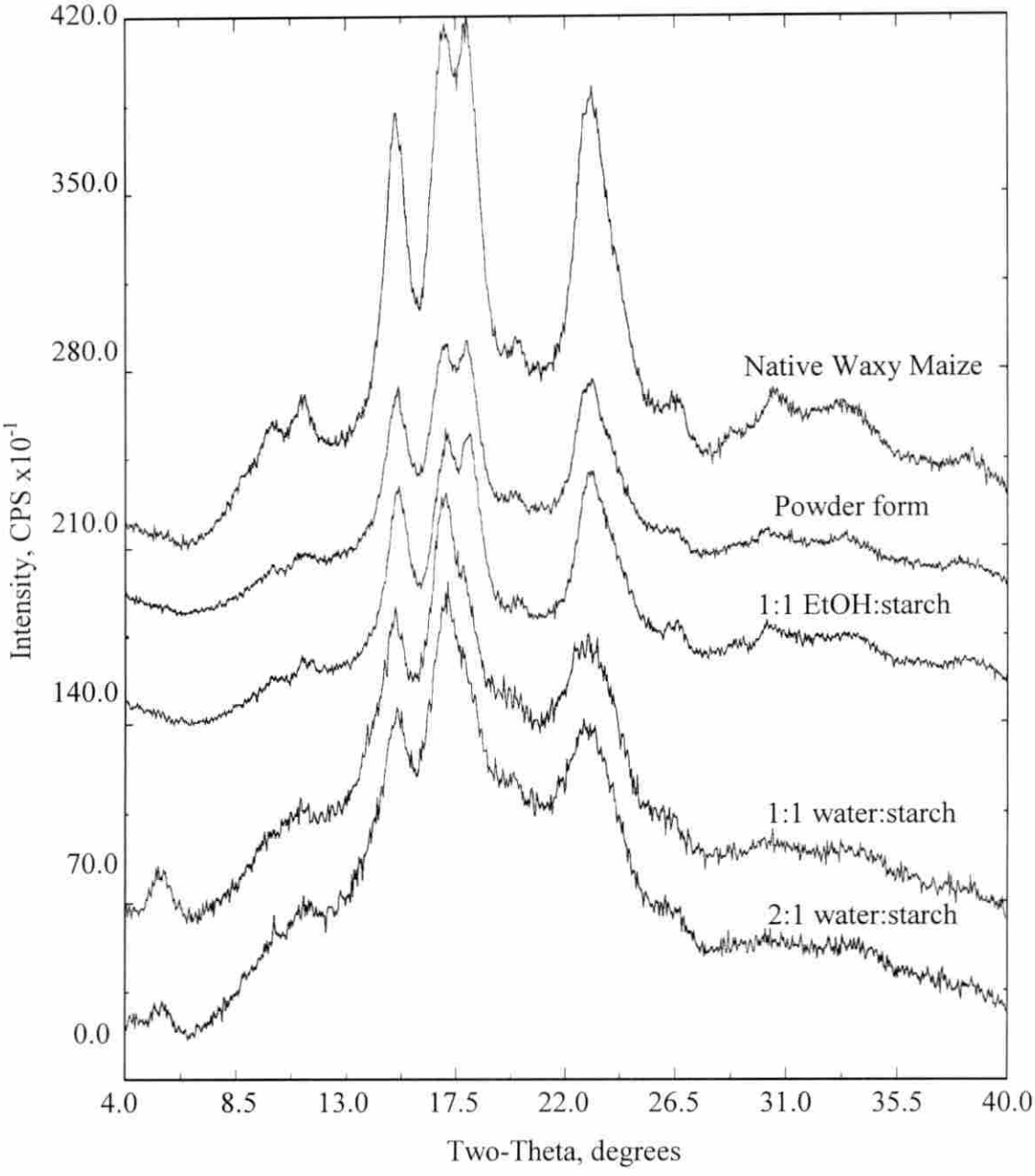
420.0
350.0
280.0
210.0
140.0
70.0
0.0
Intensity, CPS x10-1
Native Waxy Maize
Powder form
1:1 EtOH:starch
1:1 water:starch
2:1 water:starch
4.0
8.5
13.0
17.5
22.0
26.5
31.0
35.5
40.0
Two-Theta, degrees

Figure 6.

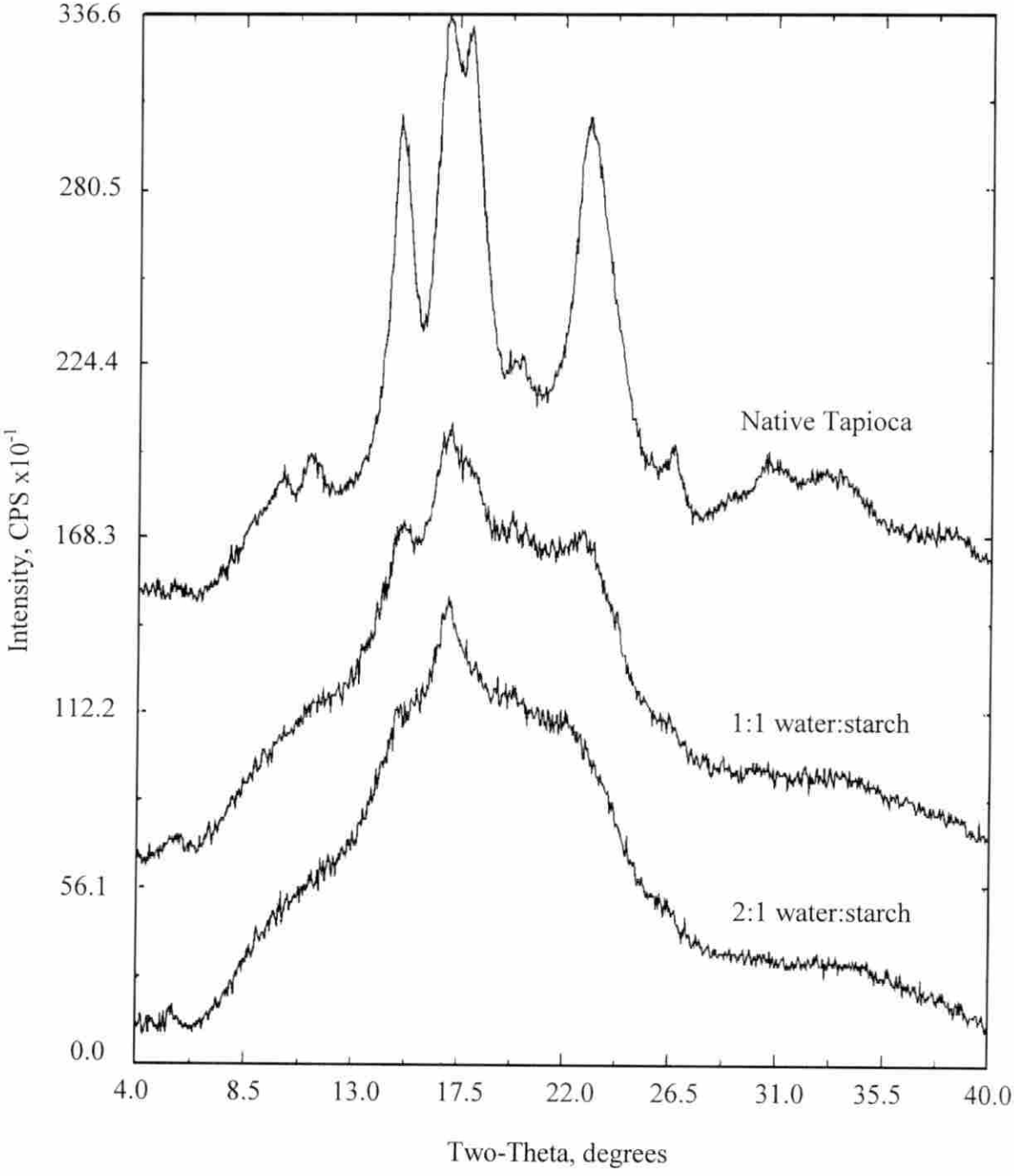
336.6
280.5
224.4
168.3
112.2
56.1
0.0
Intensity, CPS x10-1
Native Tapioca
1:1 water:starch
2:1 water:starch
4.0
8.5
13.0
17.5
22.0
26.5
31.0
35.5
40.0
Two-Theta, degrees

Figure 7.

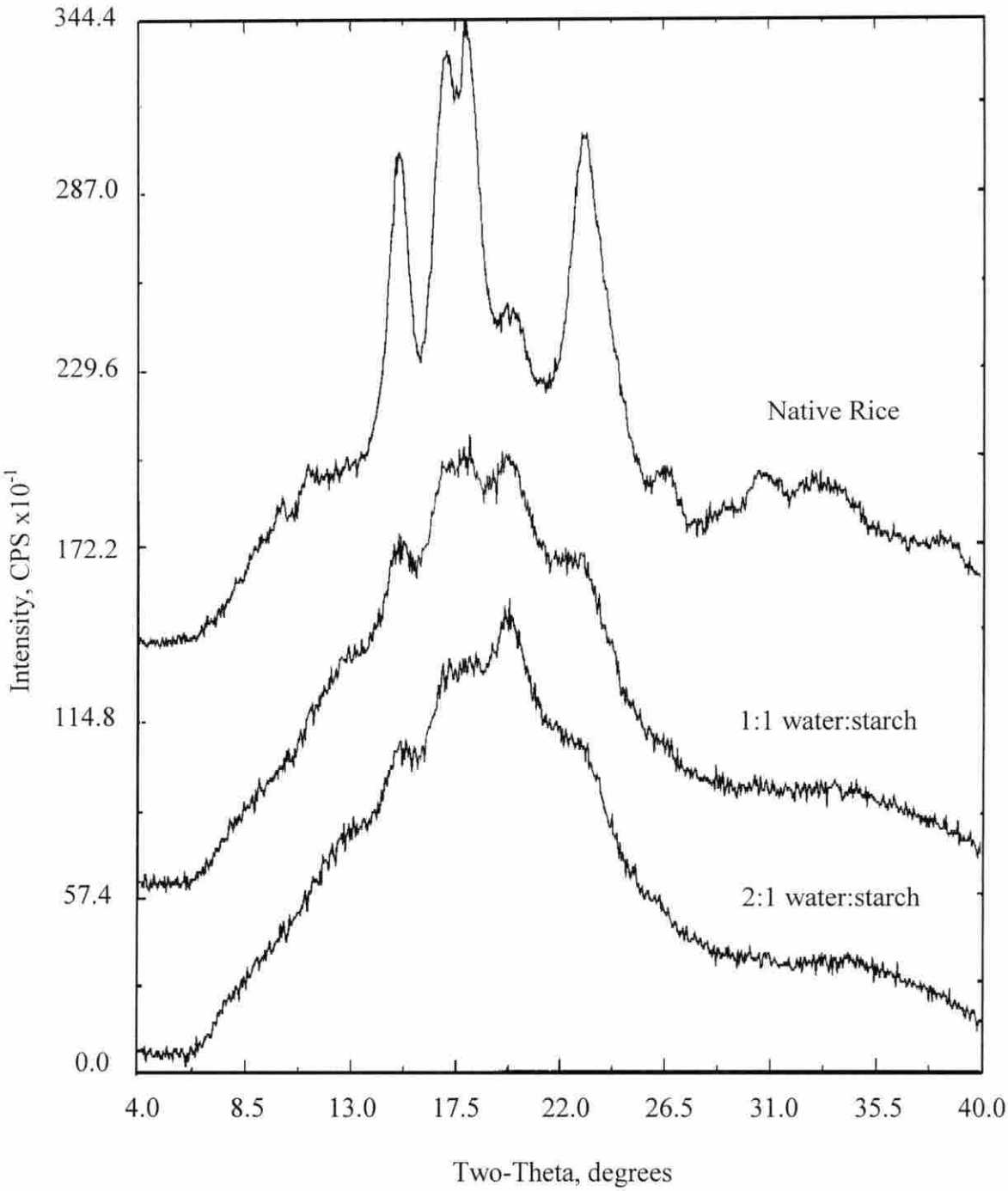
344.4
287.0
229.6
172.2
114.8
57.4
0.0
Intensity, CPS x10-1
Native Rice
1:1 water:starch
2:1 water:starch
4.0
8.5
13.0
17.5
22.0
26.5
31.0
35.5
40.0
Two-Theta, degrees

Figure 8.

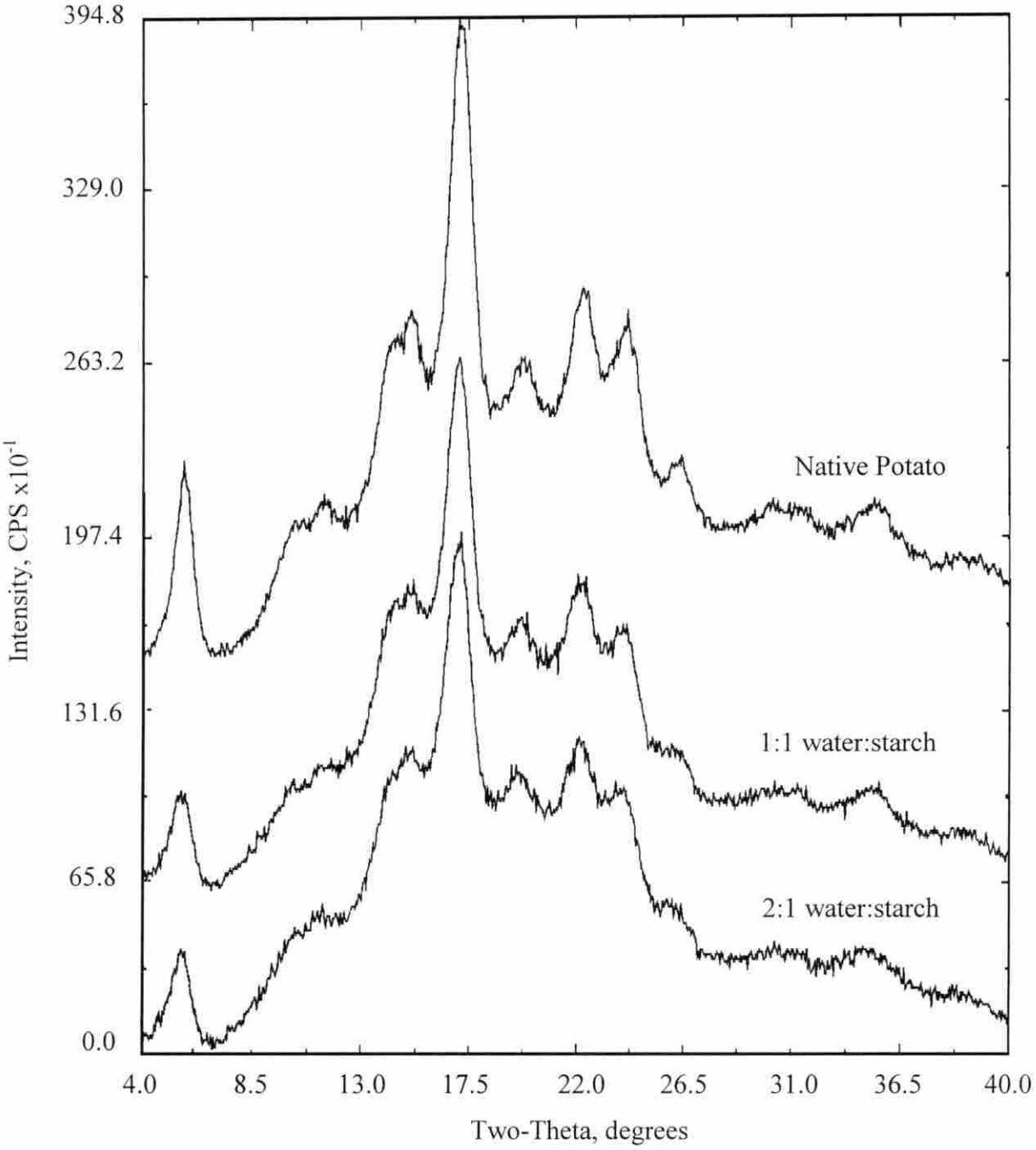
394.8
329.0
263.2
197.4
131.6
65.8
0.0
Intensity, CPS x10-1
Native Potato
1:1 water:starch
2:1 water:starch
4.0
8.5
13.0
17.5
22.0
26.5
31.0
36.5
40.0
Two-Theta, degrees

Figure 9.

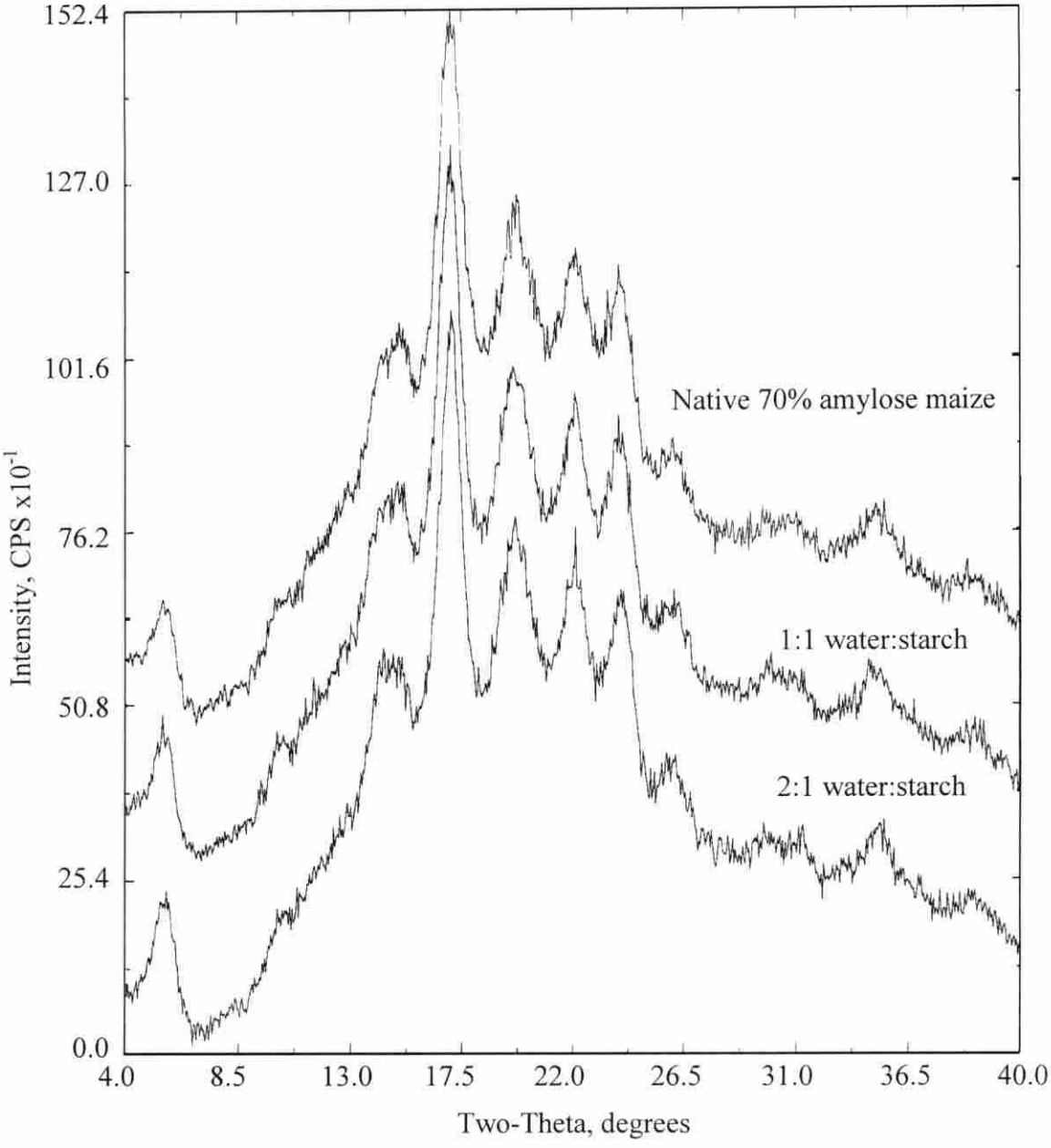
152.4
127.0
101.6
76.2
50.8
25.4
0.0
Intensity, CPS x10-1
Native 70% amylose maize
1:1 water:starch
2:1 water:starch
4.0
8.5
13.0
17.5
22.0
26.5
31.0
36.5
40.0
Two-Theta, degrees

Figure 10.

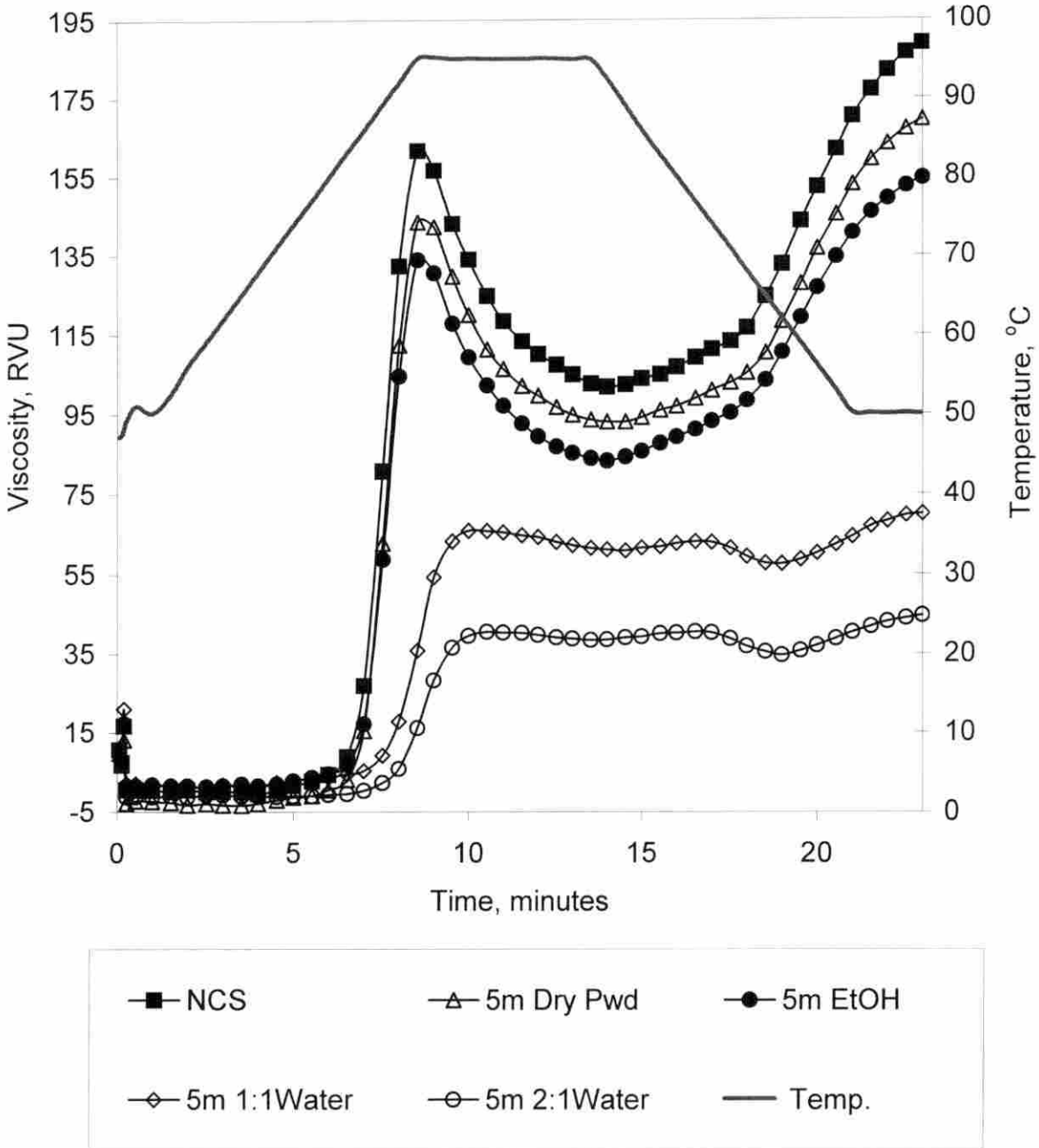
195
175
155
135
115
95
75
55
35
15
-5
Viscosity, RVU
100
90
80
70
60
50
40
30
20
10
0
Temperature, °C
0
5
10
15
20
Time, minutes
NCS
5m Dry Pwd
5m EtOH
5m 1:1Water
5m 2:1Water
Temp.

Figure 11.

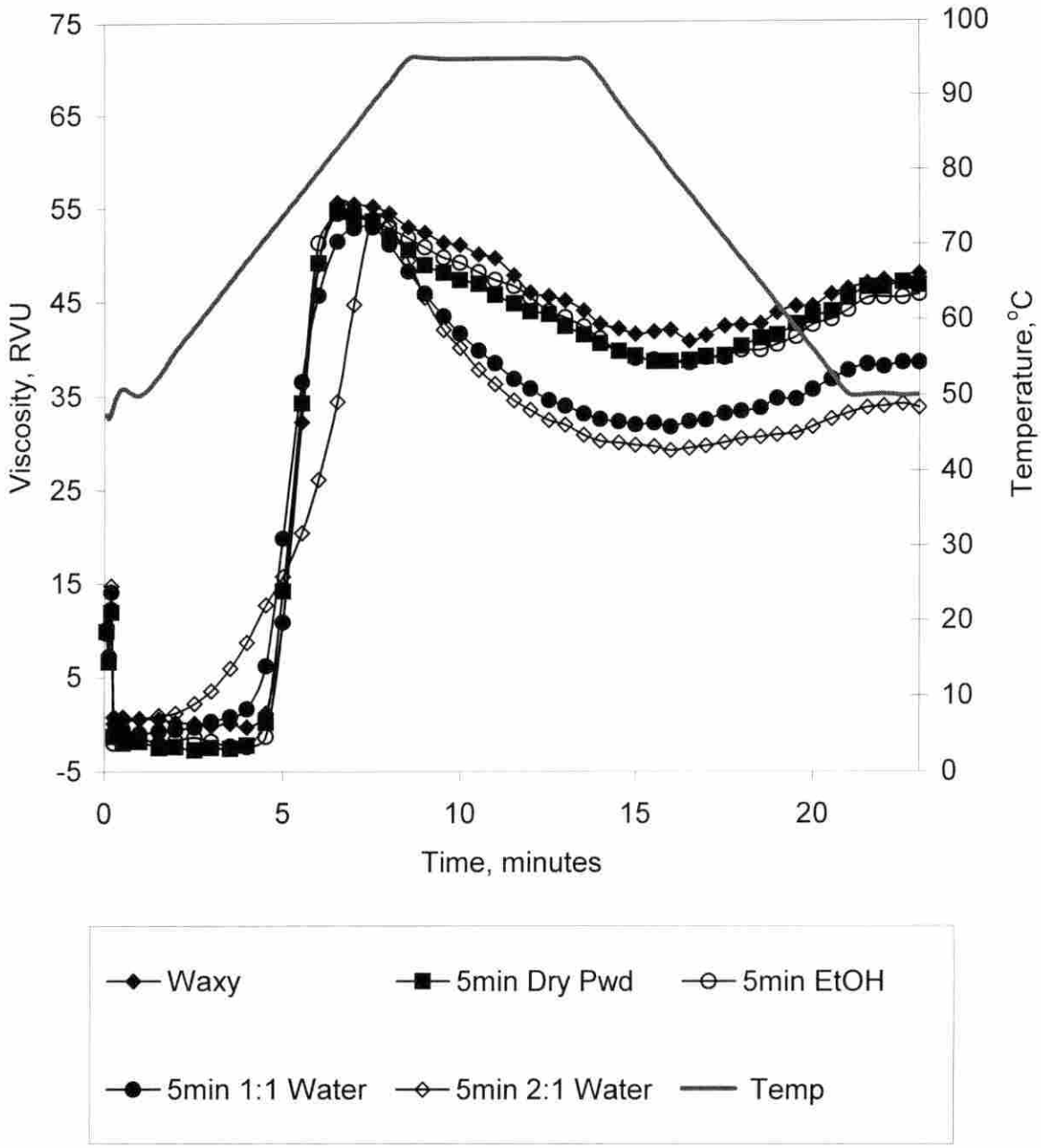
75
65
55
45
35
25
15
5
-5
Viscosity, RVU
100
90
80
70
60
50
40
30
20
10
0
Temperature, °C
0
5
10
15
20
Time, minutes
Waxy
5min Dry Pwd
5min EtOH
5min 1:1 Water
5min 2:1 Water
Temp

Figure 12.

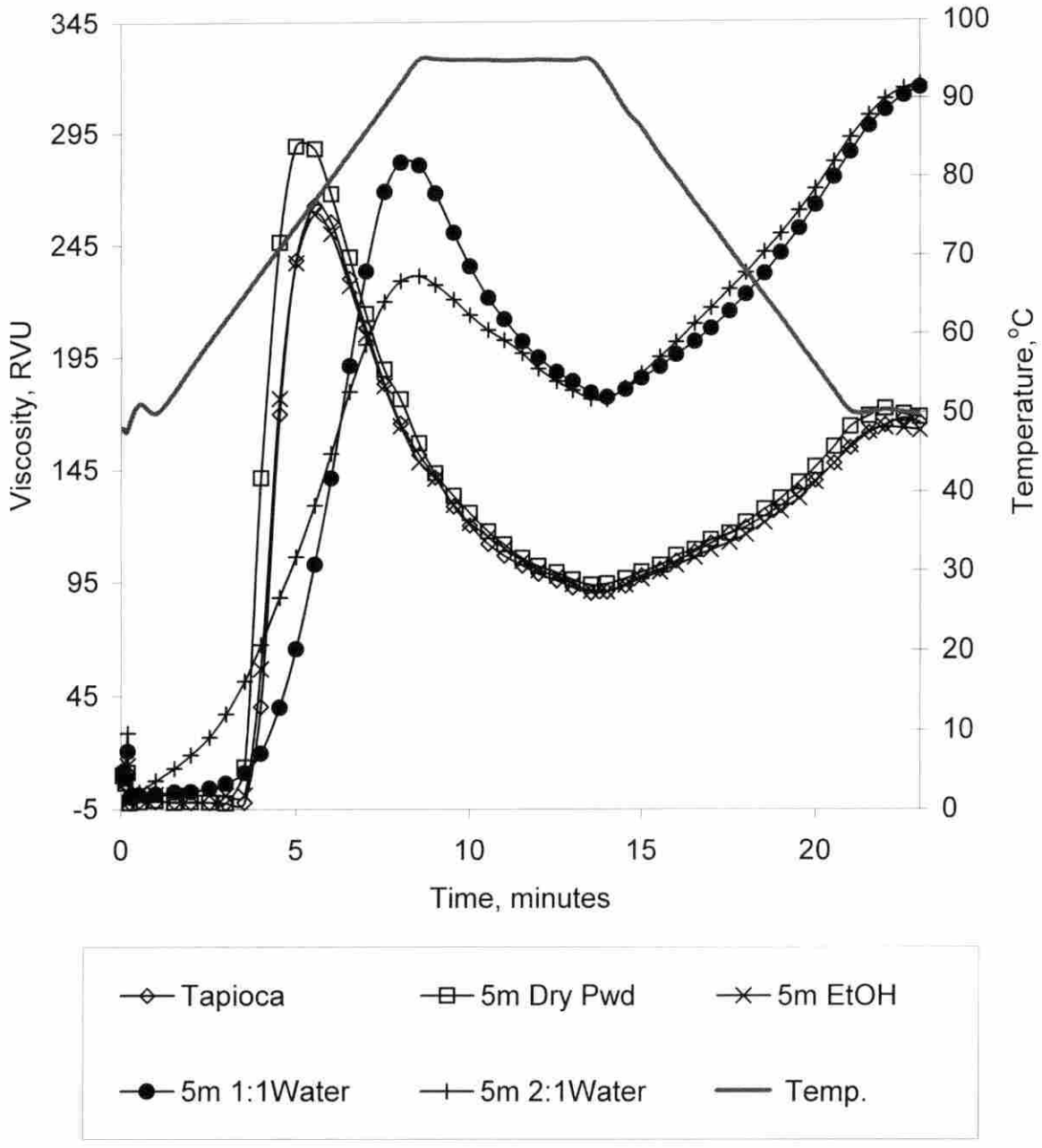
345
295
245
195
145
95
45
-5
Viscosity, RVU
100
90
80
70
60
50
40
30
20
10
0
Temperature, °C
0
5
10
15
20
Time, minutes
Tapioca
5m Dry Pwd
5m EtOH
5m 1:1Water
5m 2:1Water
Temp.

Figure 13.

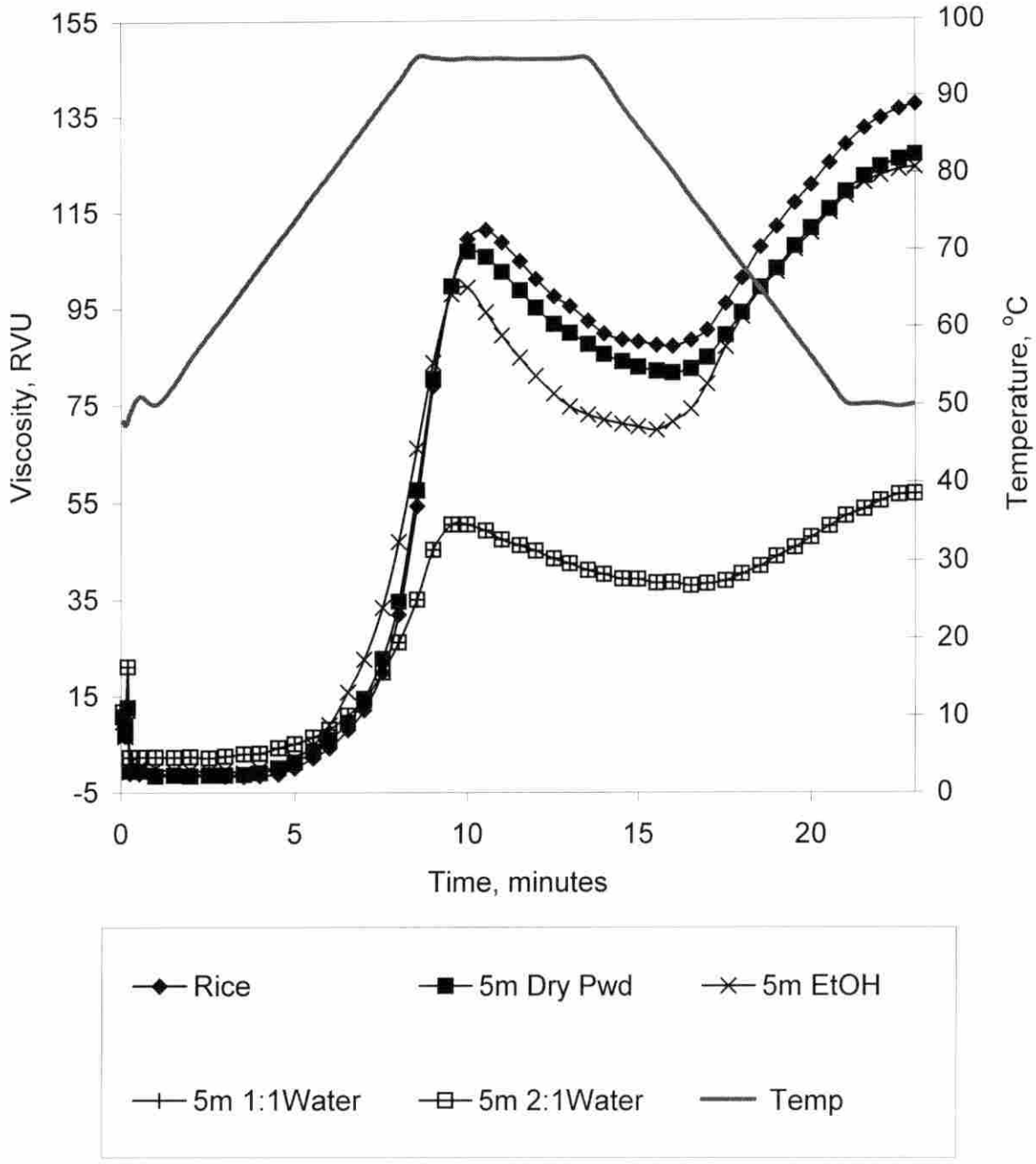
155
135
115
95
75
55
35
15
-5
Viscosity, RVU
100
90
80
70
60
50
40
30
20
10
0
Temperature, °C
0
5
10
15
20
Time, minutes
Rice
5m Dry Pwd
5m EtOH
5m 1:1Water
5m 2:1Water
Temp

Figure 14.

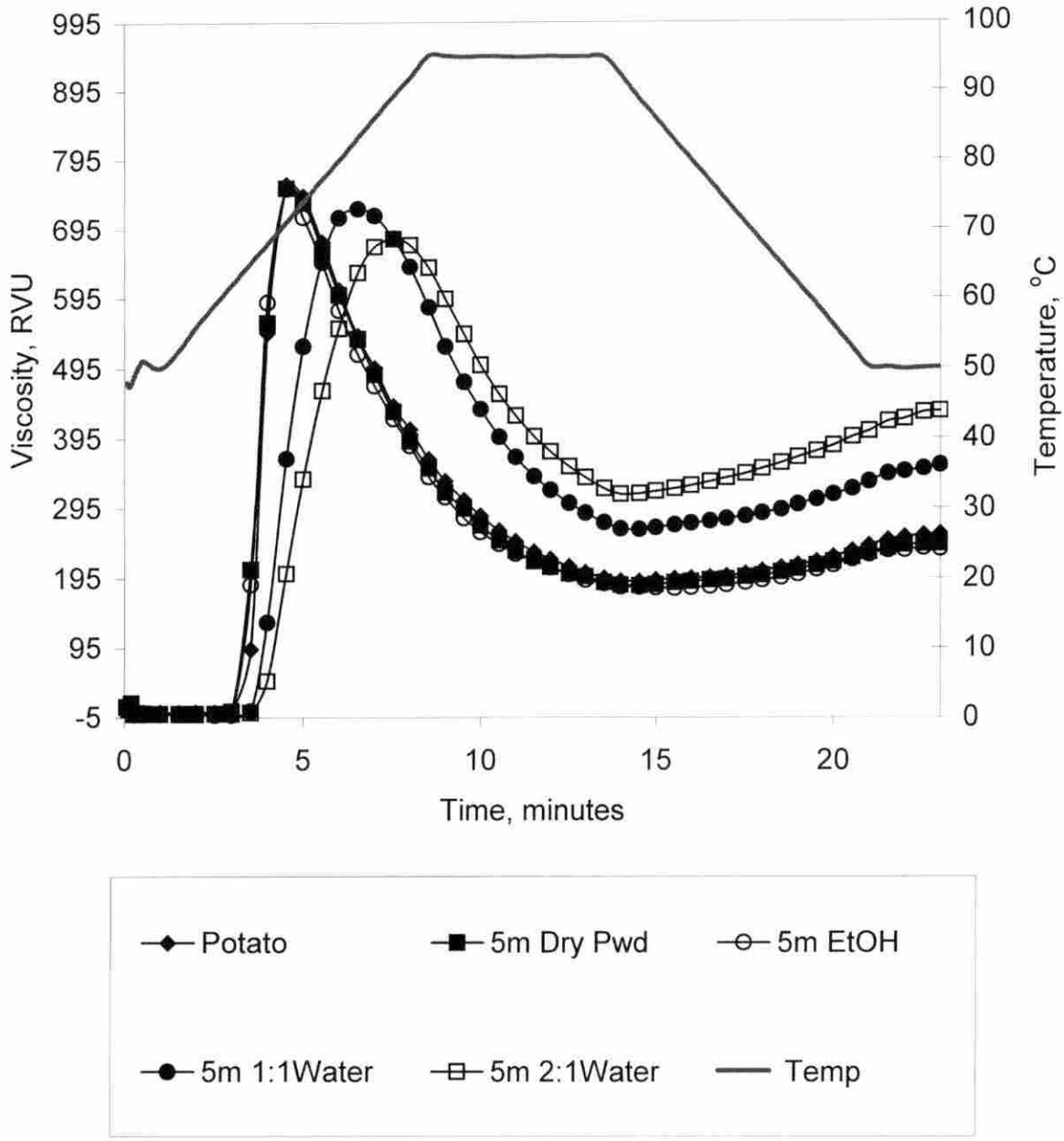
995
895
795
695
595
495
395
295
195
95
-5
Viscosity, RVU
100
90
80
70
60
50
40
30
20
10
0
Temperature, °C
0
5
10
15
20
Time, minutes
Potato
5m Dry Pwd
5m EtOH
5m 1:1Water
5m 2:1Water
Temp

Figure 15.

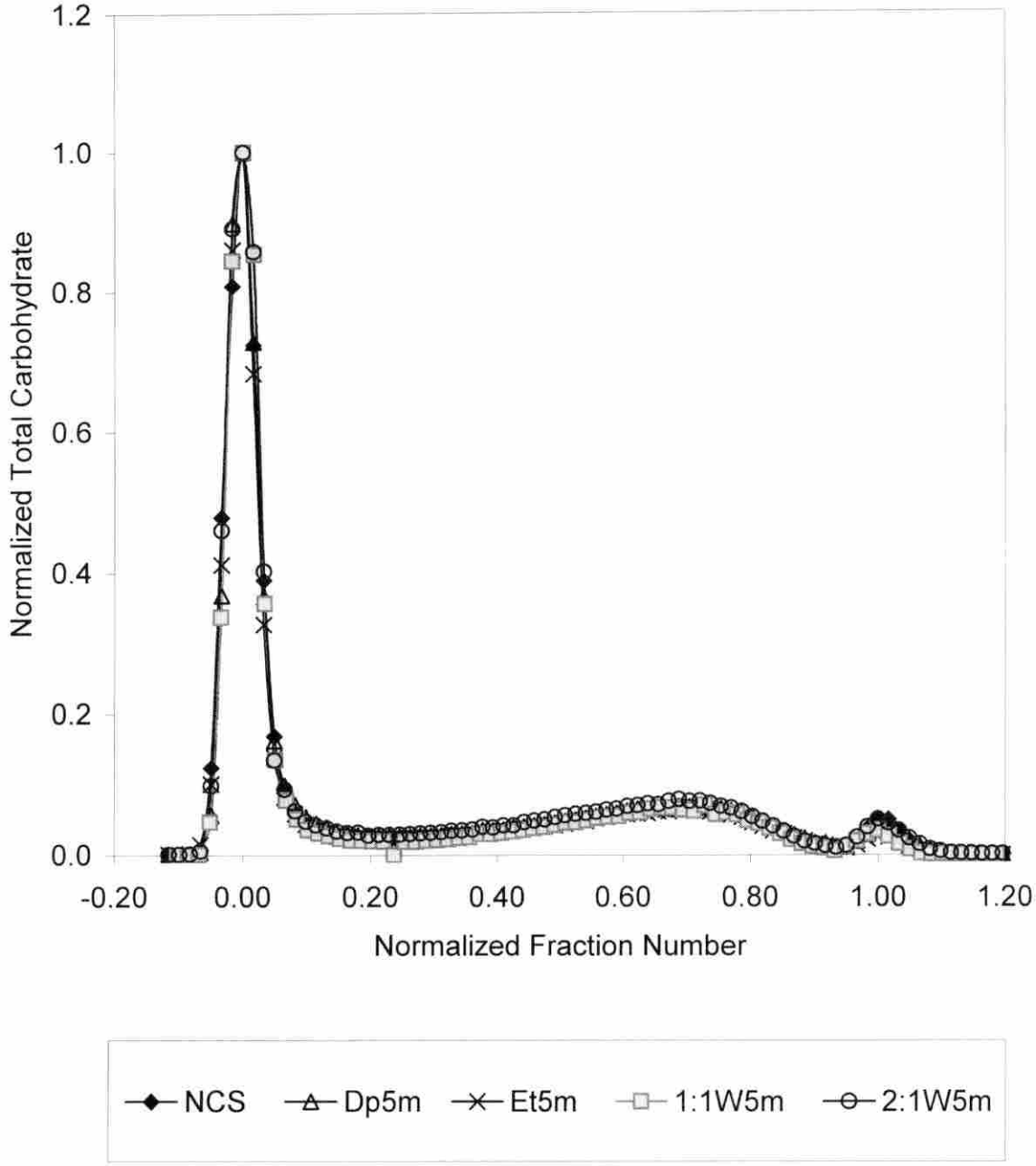
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Normalized Total Carbohydrate
-0.20
0.00
0.20
0.40
0.60
0.80
1.00
1.20
Normalized Fraction Number
NCS
Dp5m
Et5m
1:1W5m
2:1W5m

Figure 16a.

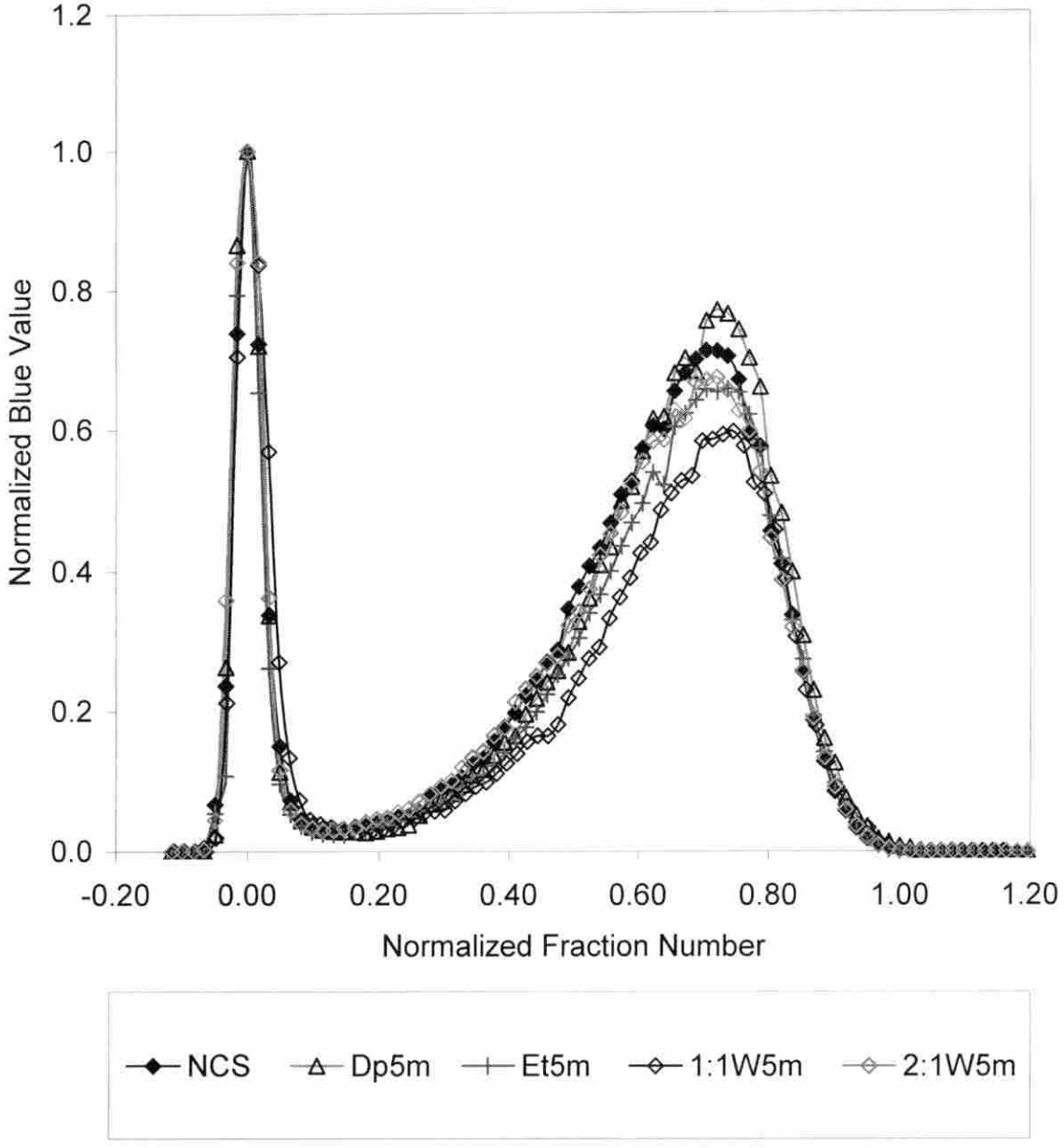
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Normalized Blue Value
-0.20
0.00
0.20
0.40
0.60
0.80
1.00
1.20
Normalized Fraction Number
NCS
Dp5m
Et5m
1:1W5m
2:1W5m

Figure 16b.

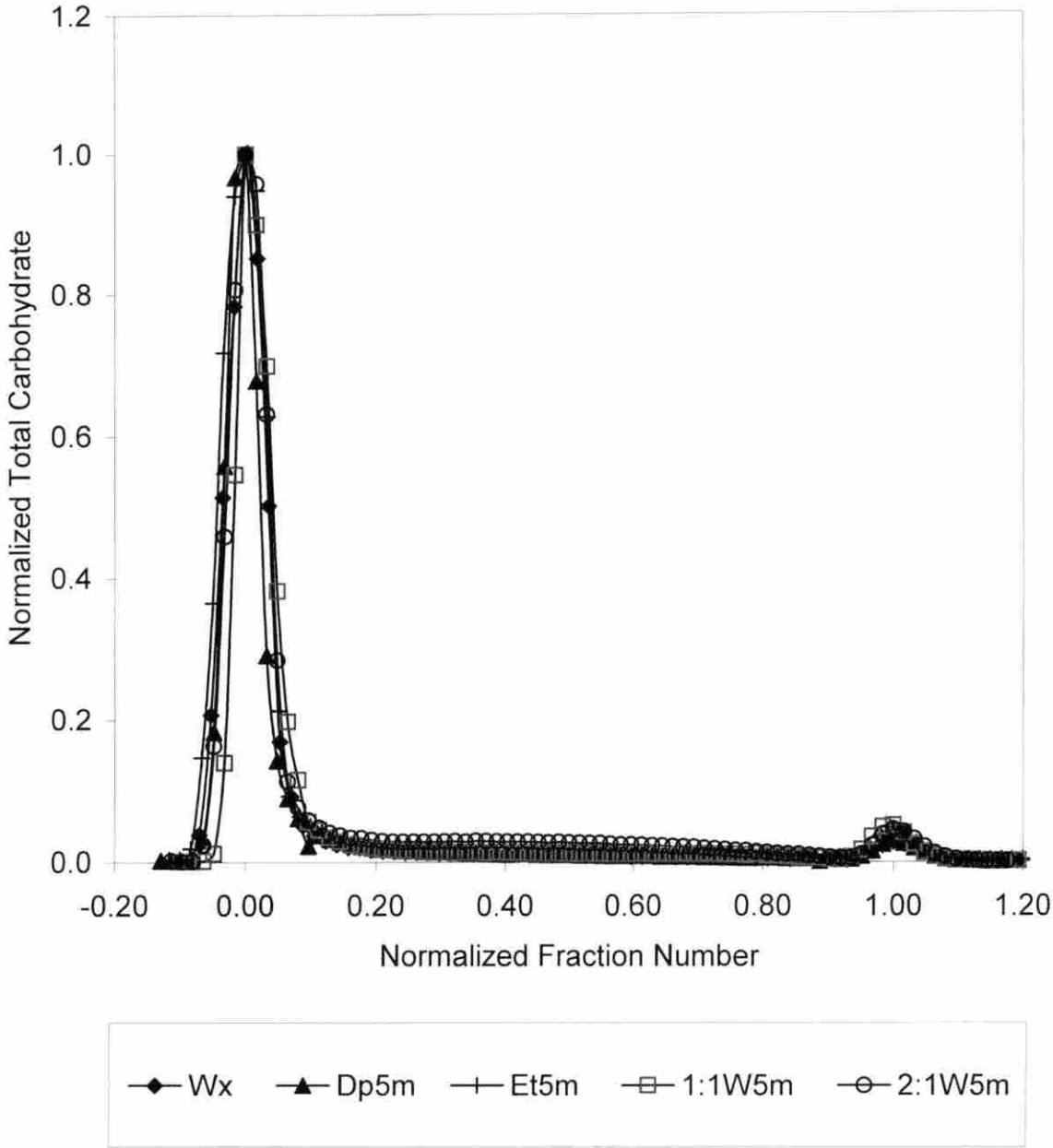
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Normalized Total Carbohydrate
-0.20
0.00
0.20
0.40
0.60
0.80
1.00
1.20
Normalized Fraction Number
Wx
Dp5m
Et5m
1:1W5m
2:1W5m

Figure 17a.

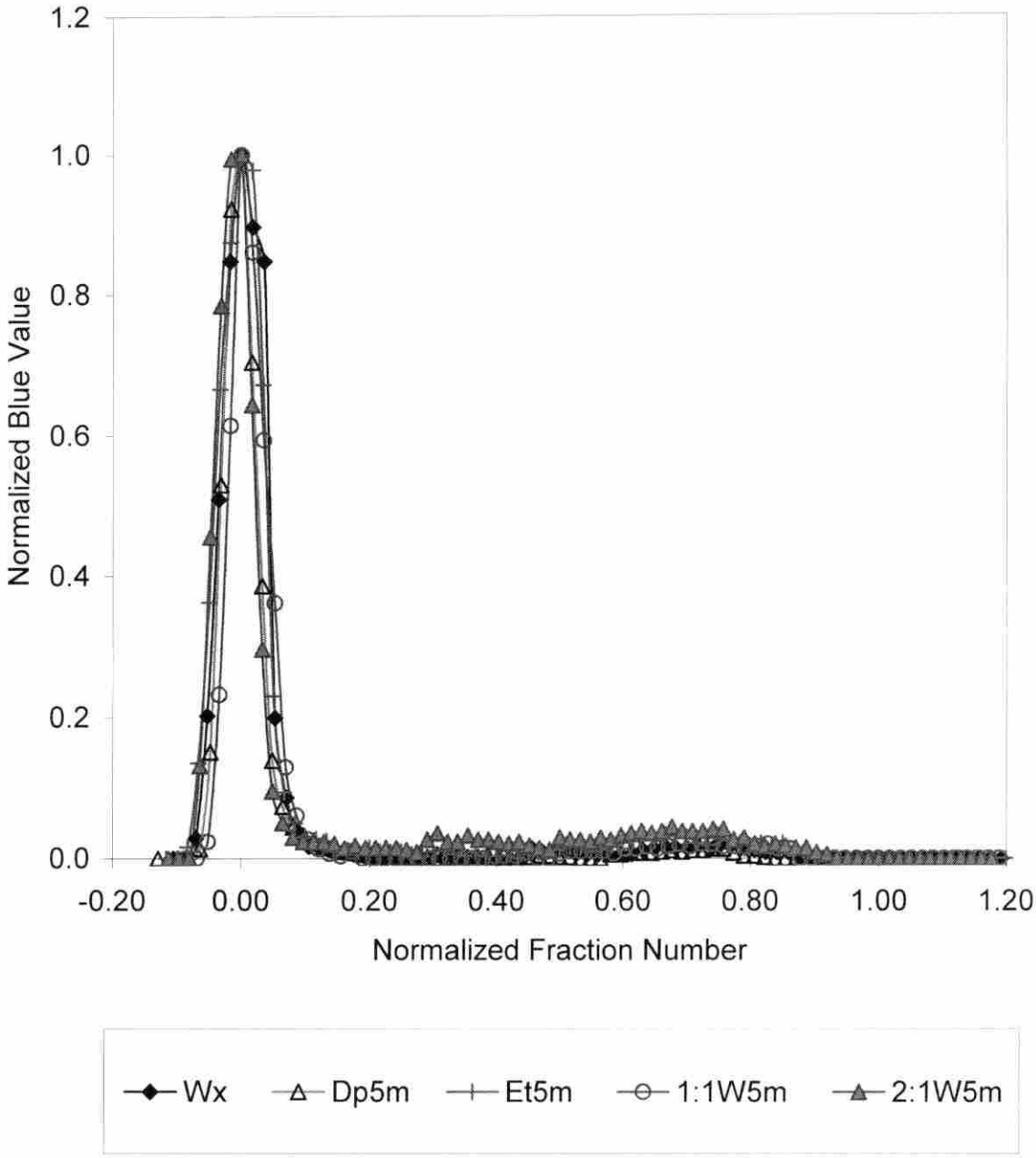
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Normalized Blue Value
-0.20
0.00
0.20
0.40
0.60
0.80
1.00
1.20
Normalized Fraction Number
Wx
Dp5m
Et5m
1:1W5m
2:1W5m

Figure 17b.

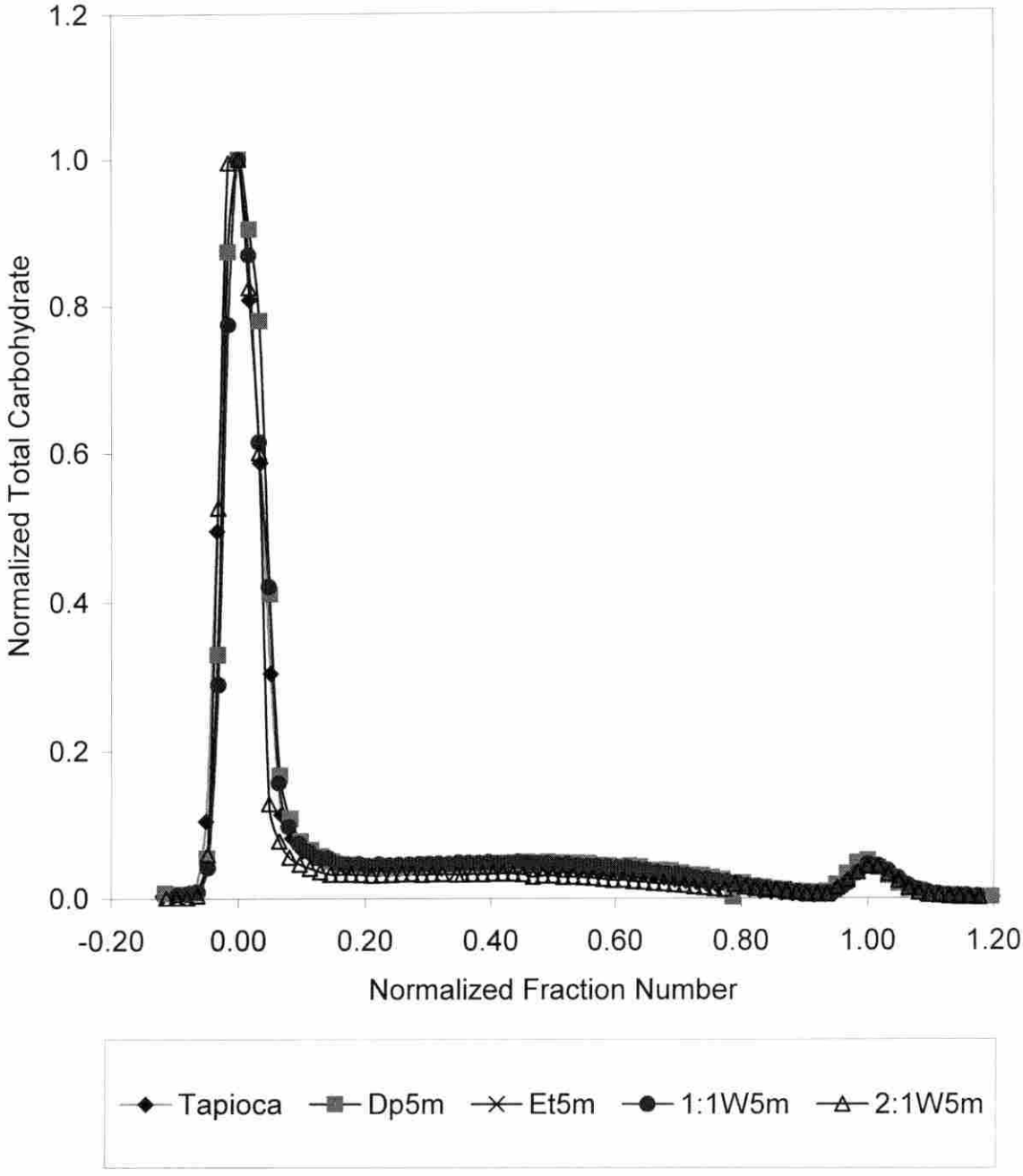
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Normalized Total Carbohydrate
-0.20
0.00
0.20
0.40
0.60
0.80
1.00
1.20
Normalized Fraction Number
Tapioca
Dp5m
Et5m
1:1W5m
2:1W5m

Figure 18a.

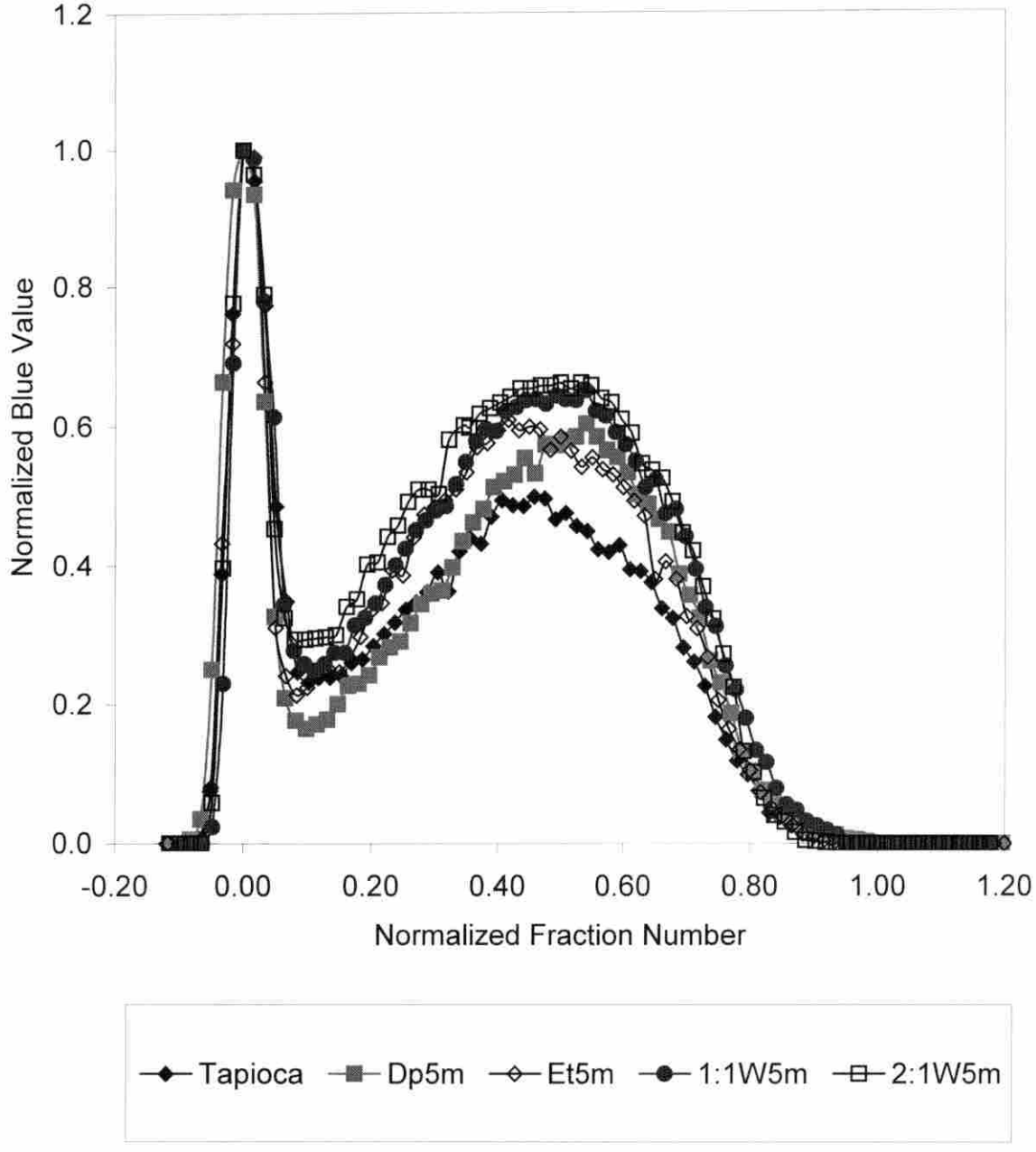
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Normalized Blue Value
-0.20
0.00
0.20
0.40
0.60
0.80
1.00
1.20
Normalized Fraction Number
Tapioca
Dp5m
Et5m
1:1W5m
2:1W5m

Figure 18b.

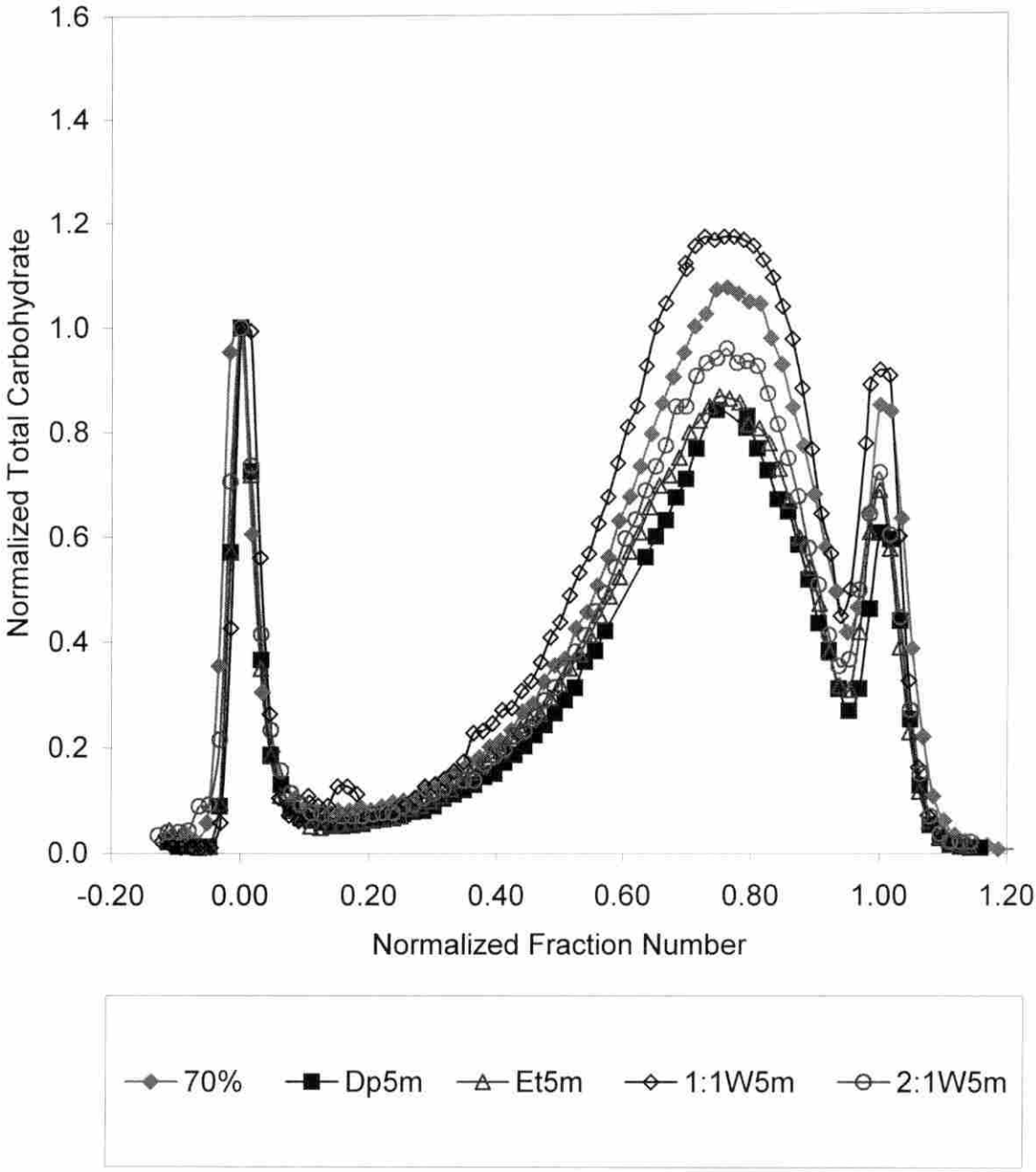
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Normalized Total Carbohydrate
-0.20
0.00
0.20
0.40
0.60
0.80
1.00
1.20
Normalized Fraction Number
70%
Dp5m
Et5m
1:1W5m
2:1W5m

Figure 19a.

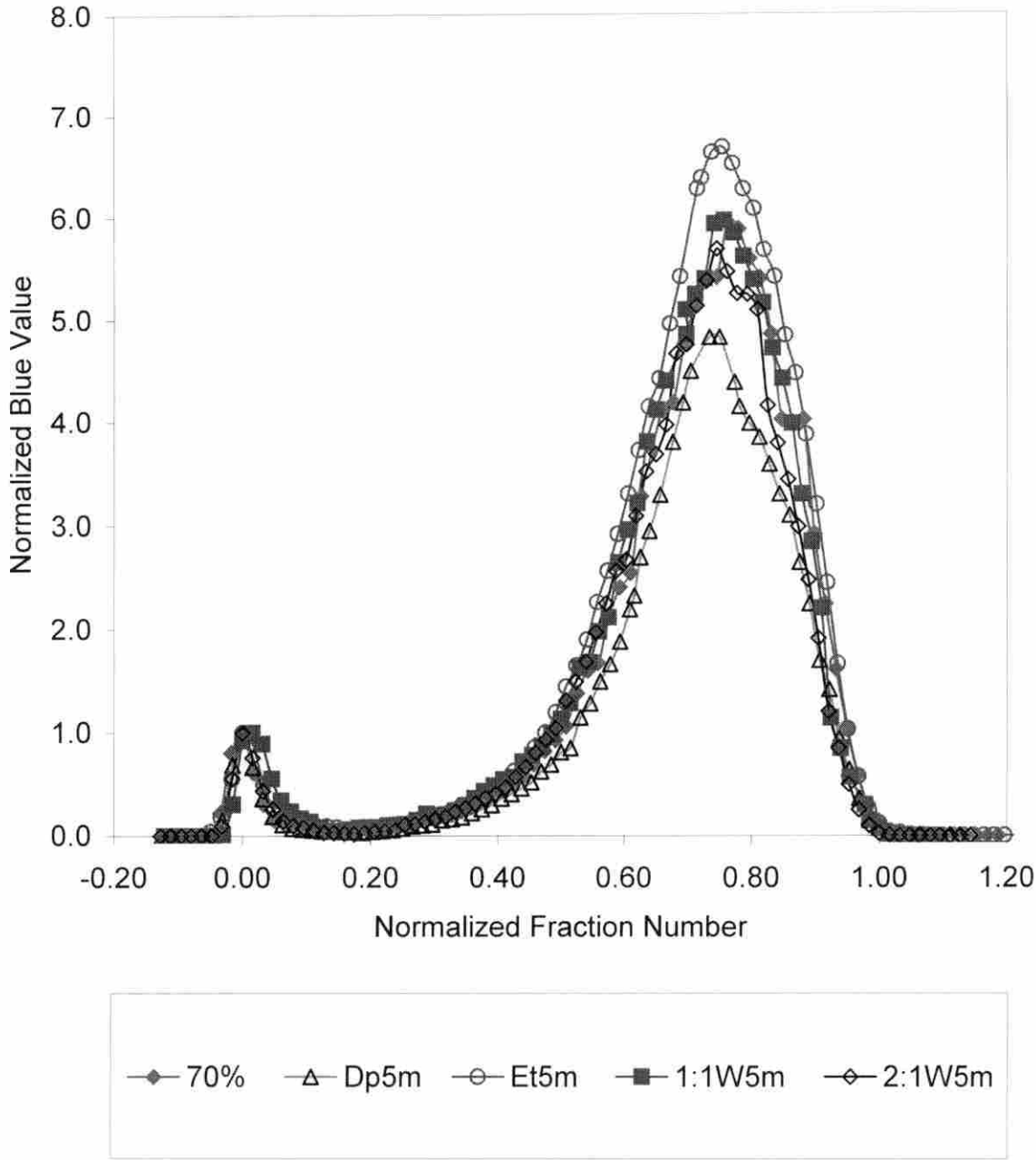
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
Normalized Blue Value
-0.20
0.00
0.20
0.40
0.60
0.80
1.00
1.20
Normalized Fraction Number
70%
Dp5m
Et5m
1:1W5m
2:1W5m

Figure 19b.

GENERAL CONCLUSIONS

The high hydrostatic pressure treatments of starch in the presence of water resulted in gelatinization at room temperature. Water is a better plasticizer than ethanol. The pasting properties of partially gelatinized starches from UHP treatment changed depending on the source of starches. Different starches gave different response to UHP treatment. There was no significant change between the treatments with 5 minutes and 1 hour dwelling time. There was no molecular degradation resulting from high hydrostatic pressure treatment. Crystalline structure changes occurred during pressure treatment. Type B X-ray pattern starches were more resistant to high pressure treatment. All methods of pressure application would at least destroy some crystallinity of starches. The dry powder compression caused more surfaces cracking, hence altering some pasting characteristics compared with the native starch.

The experiment results suggest a possibility of using HHP as a method to modify starches. The starches pressurized in the presence of water showed some resemblance with cross-linking starches. Since most of the modified starches in the market are changed with chemical reagents and most people do not like it, so a physical modification can be a new alternative if it is economically visible. In the future, a HHP unit that has a mechanism to shake the sample will be very nice to ensure uniformity of the starch suspension.

APPENDIX A. ADDITIONAL TABLES

Table 1. RVA Analysis Results for Native Maize Starch

Type	Run 1				Run 2			
	Peak (RVU)	Trough (RVU)	Final Visc (RVU)	Pasting Temp (oC)	Peak (RVU)	Trough (RVU)	Final Visc (RVU)	Pasting Temp (oC)
Native I	161.33	103.58	184.83	83.95				
Native II	162.00	101.58	188.75	83.50				
100ksi,5min,Dry Pwd I	160.67	102.42	185.42	83.90	143.17	88.25	165.50	84.30
100ksi,5min,Dry Pwd II	159.67	96.92	181.92	83.95	145.75	92.50	169.50	84.35
100ksi,1hr,Dry Pwd I	157.83	90.58	180.75	83.15	142.42	84.33	164.75	84.30
100ksi,1hr,Dry Pwd II	160.58	100.08	186.25	83.90	143.25	85.33	165.17	84.35
100ksi,5min,in EtOH I	152.17	91.83	172.50	81.55	134.25	83.92	155.92	84.75
100ksi,5min,in EtOH II	151.58	92.50	172.00	81.55	135.75	82.83	154.50	84.30
100ksi,1hr,in EtOH I	148.83	91.00	168.33	80.70	140.83	96.75	165.33	84.40
100ksi,1hr,in EtOH II	147.50	90.75	166.08	81.15	140.83	92.83	165.50	83.90
100ksi,5min,1:1 water I	76.17	63.42	79.25	89.55	65.67	56.58	69.75	90.35
100ksi,5min,1:1 water II	75.58	63.58	79.67	89.95	65.83	57.25	70.00	89.60
100ksi,1hr,1:1 water I	77.42	64.50	77.92	89.10	64.75	56.00	68.75	91.20
100ksi,1hr,1:1 water II	77.17	63.58	77.33	89.55	62.00	52.33	64.50	89.55
100ksi,5min,2:1 water I	46.58	40.50	50.83	92.70	42.33	36.92	47.17	91.90
100ksi,5min,2:1 water II	47.25	41.83	51.75	92.70	40.67	34.58	44.58	94.00
100ksi,1hr,2:1 water I	34.92	29.75	36.50	94.35	36.50	31.42	39.25	93.95
100ksi,1hr,2:1 water II	35.50	31.17	38.67	92.70	38.17	33.83	42.08	93.55

Table 2. RVA Analysis Results for Waxy Maize Starch

Type	Run 1				Run 2			
	Peak (RVU)	Trough (RVU)	Final Visc (RVU)	Pasting Temp (oC)	Peak (RVU)	Trough (RVU)	Final Visc (RVU)	Pasting Temp (oC)
Native I	56.08	40.75	47.83	72.25				
Native II	56.00	39.67	46.08	72.35				
100ksi,5min,Dry Pwd I	51.17	37.50	44.25	71.90	54.92	38.17	46.58	71.15
100ksi,5min,Dry Pwd II	53.58	37.58	44.25	70.75	56.33	39.42	46.53	71.50
100ksi,1hr,Dry Pwd I	51.25	36.08	42.67	71.55	56.08	39.42	45.92	71.95
100ksi,1hr,Dry Pwd II	49.25	35.08	42.67	72.00	55.83	39.75	47.83	71.50
100ksi,5min,in EtOH I	50.58	33.50	38.25	72.30	54.92	37.67	45.67	72.35
100ksi,5min,in EtOH II	49.75	32.33	37.83	72.35	56.17	40.50	47.92	72.30
100ksi,1hr,in EtOH I	50.08	34.25	37.92	72.35	54.00	39.17	45.42	71.95
100ksi,1hr,in EtOH II	50.08	33.83	37.83	72.30	56.83	39.67	46.17	71.90
100ksi,5min,1:1 water I	37.58	23.17	28.17	72.35	53.33	31.67	38.42	71.15
100ksi,5min,1:1 water II	39.67	23.92	27.50	73.15	52.42	31.00	37.00	71.95
100ksi,1hr,1:1 water I	42.75	26.42	31.75	72.35	54.50	32.50	38.42	71.95
100ksi,1hr,1:1 water II	48.58	29.42	35.50	72.35	54.83	33.08	39.08	71.50
100ksi,5min,2:1 water I	29.25	21.25	25.08	50.23	54.58	29.08	33.67	50.00
100ksi,5min,2:1 water II	29.92	21.25	25.50	51.33	54.33	28.50	33.50	51.25
100ksi,1hr,2:1 water I	44.00	25.33	30.67	50.45	59.58	29.08	34.42	50.02
100ksi,1hr,2:1 water II	42.25	24.75	30.00	50.35	60.33	29.25	34.92	50.00

Table 3. RVA Analysis Results for Tapioca Starch

Type	Run 1				Run 2			
	Peak (RVU)	Trough (RVU)	Final Visc (RVU)	Pasting Temp (oC)	Peak (RVU)	Trough (RVU)	Final Visc (RVU)	Pasting Temp (oC)
Native I	263.00	88.42	165.50	66.35				
Native II	265.83	88.83	167.58	66.40				
100ksi,5min,Dry Pwd I	293.67	90.33	167.17	63.60	292.67	92.25	168.00	63.90
100ksi,5min,Dry Pwd II	291.42	90.50	156.08	63.50	297.75	92.33	170.25	63.50
100ksi,1hr,Dry Pwd I	287.83	91.92	169.25	63.55	292.25	93.92	167.50	64.30
100ksi,1hr,Dry Pwd II	291.50	93.50	167.92	63.95	290.92	91.42	162.92	63.90
100ksi,5min,in EtOH I	246.83	89.17	159.25	66.40	258.83	89.92	162.08	65.95
100ksi,5min,in EtOH II	252.08	90.25	160.83	65.95	261.58	89.25	164.83	66.35
100ksi,1hr,in EtOH I	267.92	93.58	166.08	66.35	264.17	92.08	165.08	65.95
100ksi,1hr,in EtOH II	267.83	95.50	167.00	65.95	259.25	91.67	163.50	66.00
100ksi,5min,1:1 water I	269.00	140.58	254.00	63.60	282.92	176.83	314.67	66.30
100ksi,5min,1:1 water II	269.17	140.00	254.00	50.00	280.00	174.08	310.33	66.35
100ksi,1hr,1:1 water I	209.25	170.08	280.92	67.50	289.83	187.92	319.83	67.90
100ksi,1hr,1:1 water II	208.50	167.58	275.17	68.35	286.75	182.25	312.42	66.00
100ksi,5min,2:1 water I	158.33	148.67	276.50	51.60	231.33	174.67	316.25	57.15
100ksi,5min,2:1 water II	152.58	148.25	277.25	56.65	230.58	173.83	315.33	58.30
100ksi,1hr,2:1 water I	157.83	157.75	279.58	50.40	160.00	154.58	274.42	59.50
100ksi,1hr,2:1 water II	155.67	155.33	272.75	50.56	160.00	153.67	274.25	56.35

Table 4. RVA Analysis Results for Rice Starch

Type	Run 1				Run 2			
	Peak (RVU)	Trough (RVU)	Final Visc (RVU)	Pasting Temp (oC)	Peak (RVU)	Trough (RVU)	Final Visc (RVU)	Pasting Temp (oC)
Native I	113.08	86.92	135.75	88.00				
Native II	111.67	86.67	137.17	89.55				
100ksi,5min,Dry Pwd I	111.67	83.75	131.75	88.75	104.83	80.92	125.42	87.50
100ksi,5min,Dry Pwd II	110.42	83.42	133.42	86.75	107.08	81.50	126.67	88.30
100ksi,1hr,Dry Pwd I	106.50	78.92	125.92	88.35	105.17	78.25	126.50	85.60
100ksi,1hr,Dry Pwd II	106.92	79.17	127.33	88.70	107.08	82.17	128.92	87.95
100ksi,5min,in EtOH I	105.75	77.17	130.92	81.55	100.50	69.33	124.75	83.95
100ksi,5min,in EtOH II	105.67	76.08	130.75	82.75	100.00	69.75	124.08	85.10
100ksi,1hr,in EtOH I	104.08	75.50	128.83	87.55	101.92	72.67	127.08	83.50
100ksi,1hr,in EtOH II	105.00	77.75	130.00	84.70	99.17	70.00	123.83	87.50
100ksi,5min,1:1 water I	58.08	43.17	66.75	90.70	52.75	39.42	58.42	91.15
100ksi,5min,1:1 water II	59.00	43.67	65.75	87.05	50.83	37.83	56.58	91.15
100ksi,1hr,1:1 water I	61.25	46.00	68.83	90.30	55.25	41.83	62.17	94.00
100ksi,1hr,1:1 water II	62.17	46.33	69.92	90.20	54.08	42.42	64.08	93.60
100ksi,5min,2:1 water I	67.50	54.67	78.08	90.40	51.83	38.58	57.83	93.55
100ksi,5min,2:1 water II	67.75	55.42	79.92	87.95	49.42	36.83	55.50	95.60
100ksi,1hr,2:1 water I	60.17	48.25	70.67	93.55	40.33	29.92	46.92	95.55
100ksi,1hr,2:1 water II	61.67	49.67	70.75	89.10	43.58	31.08	47.17	93.69

Table 5. RVA Analysis Results for Potato Starch

Type	Run 1				Run 2			
	Peak (RVU)	Trough (RVU)	Final Visc (RVU)	Pasting Temp (oC)	Peak (RVU)	Trough (RVU)	Final Visc (RVU)	Pasting Temp (oC)
Native I	759.58	189.58	258.50	63.55				
Native II	760.58	189.67	256.17	63.55				
100ksi,5min,Dry Pwd I	774.17	197.25	258.42	62.05	760.25	188.17	247.17	61.55
100ksi,5min,Dry Pwd II	781.67	192.83	263.00	61.95	762.67	184.17	244.17	61.55
100ksi,1hr,Dry Pwd I	775.08	188.08	258.92	61.60	758.67	184.33	244.42	61.95
100ksi,1hr,Dry Pwd II	777.08	192.25	259.58	61.60	765.83	186.50	242.00	61.65
100ksi,5min,in EtOH I	742.25	179.50	238.25	62.35	747.42	169.17	240.42	61.95
100ksi,5min,in EtOH II	736.42	171.17	238.58	62.75	753.58	180.33	237.08	62.40
100ksi,1hr,in EtOH I	748.33	175.33	233.42	62.75	754.50	174.25	240.00	62.75
100ksi,1hr,in EtOH II	744.75	175.58	236.25	62.75	754.92	168.25	240.25	62.35
100ksi,5min,1:1 water I	871.50	206.17	292.08	64.35	730.92	267.50	360.08	65.15
100ksi,5min,1:1 water II	870.42	204.75	302.75	64.35	727.50	264.92	356.42	64.70
100ksi,1hr,1:1 water I	769.33	215.08	308.75	65.10	722.17	259.08	367.17	65.55
100ksi,1hr,1:1 water II	775.83	215.67	305.25	65.55	719.58	260.33	366.17	65.10
100ksi,5min,2:1 water I	467.92	292.67	438.83	64.75	675.42	313.67	433.17	65.90
100ksi,5min,2:1 water II	468.83	289.58	428.08	64.35	680.92	314.83	433.33	65.15
100ksi,1hr,2:1 water I	582.58	302.08	430.58	66.75	606.50	325.75	458.17	67.10
100ksi,1hr,2:1 water II	587.58	299.67	439.00	65.95	603.58	339.50	475.25	66.35

Table 6. The Thermal properties of 5 min HHP treated A-type starches determined by Differential Scanning Calorimetry (trial 2)

	Peak 1				Peak 2			
Starch Type	**To[a] (°C)**	**Tp[b] (°C)**	**Tc[c] (°C)**	**ΔH[d] (J/g)**	**To (°C)**	**Tp (°C)**	**Tc (°C)**	**ΔH (J/g)**
Normal Maize:								
Untreated	ND	ND	ND	ND	66.0±0.1[sd]	70.1±0.2	80.2±0.0	14.0±0.7
in powder form	ND	ND	ND	ND	61.7±0.2	67.6±0.1	78.5±0.2	11.7±0.1
in EtOH (1:1)	ND	ND	ND	ND	65.4±0.3	69.2±0.2	78.7±0.3	13.6±0.2
in H_2O (1:1)	45.0±2.3	52.3±1.1	61.3±2.2	0.6±0.1	66.1±0.3	71.0±0.2	79.3±1.2	0.8±0.2
in H_2O (1:1), Di	44.6±0.1	49.6±1.7	59.8±1.5	0.4±0.2	65.4±0.4	71.1±0.7	78.9±1.0	1.5±0.0
in H_2O (2:1)	42.9±1.8	51.2±0.6	64.5±2.1	0.4±0.1	ND	ND	ND	ND
in H_2O (2:1), Di	ND	ND	ND	ND	ND	ND	ND	ND
Waxy Maize:								
Untreated	ND	ND	ND	ND	64.6±0.2[sd]	70.4±0.3	81.2±0.4	16.1±0.2
in powder form	ND	ND	ND	ND	62.1±0.4	68.7±0.2	81.4±2.0	15.0±0.3
in EtOH (1:1)	ND	ND	ND	ND	64.1±0.1	69.8±0.4	81.4±0.4	16.1±0.1
in H_2O (1:1)	43.7±1.2	51.2±0.4	61.1±0.7	1.3±0.1	65.5±0.4	71.5±0.3	81.6±0.4	2.9±0.0
in H_2O (1:1), Di	45.6±0.2	52.2±0.1	58.4±2.6	0.3±0.4	65.3±0.8	72.5±1.0	83.2±3.0	3.2±2.4
in H_2O (2:1)	42.0±0.4	50.0±0.6	70.4±0.1	5.4±0.5	ND	ND	ND	ND
in H_2O (2:1), Di	ND	ND	ND	ND	ND	ND	ND	ND
Tapioca:								
Untreated	ND	ND	ND	ND	64.9±0.1[sd]	69.1±0.1	82.2±0.5	14.8±0.3
in powder form	ND	ND	ND	ND	56.9±0.1	63.4±0.1	75.5±0.5	12.7±0.2
in EtOH (1:1)	ND	ND	ND	ND	61.7±0.0	66.0±0.0	79.0±0.7	14.8±0.5
in H_2O (1:1)	ND	ND	ND	ND	60.9±0.5	68.1±0.3	75.2±0.7	1.7±0.2
in H_2O (1:1), Di	44.9±0.3	49.5±2.4	59.1±2.4	0.3±0.0	62.8±0.7	69.2±0.2	78.1±1.3	1.5±0.1
in H_2O (2:1)	42.9±1.8	47.2±0.6	54.2±1.7	0.3±0.1	ND	ND	ND	ND
in H_2O (2:1), Di	ND	ND	ND	ND	ND	ND	ND	ND

a,b,c,d are onset, peak,and completion temperature, and enthalpy change, respectively.

sd is standard deviation. ND is not detected. Di is directly after pressure treatment.

Table 6. (continued)

Starch Type	Peak 1				Peak 2			
	To[a] (°C)	Tp[b] (°C)	Tc[c] (°C)	ΔH[d] (J/g)	To (°C)	Tp (°C)	Tc (°C)	ΔH (J/g)
Rice:								
Untreated	ND	ND	ND	ND	60.6±0.6[sd]	68.3±0.1	84.7±2.3	16.8±4.1
in powder form	ND	ND	ND	ND	56.4±0.2	66.3±0.2	82.7±0.8	12.1±0.6
in EtOH (1:1)	ND	ND	ND	ND	60.5±0.3	68.2±0.3	83.1±1.0	14.4±1.0
in H_2O (1:1)	44.8±0.0	50.5±0.0	54.1±0.0	0.2±0.0	63.8±0.5	73.1±0.5	83.0±0.2	1.7±0.2
in H_2O (1:1), Di	ND	ND	ND	ND	67.1±0.5	77.2±0.5	81.9±1.0	0.3±0.2
in H_2O (2:1)	ND	ND	ND	ND	64.8±0.4	73.2±0.3	82.1±1.0	1.8±0.2
in H_2O (2:1), Di	46.9±0.0	50.7±0.0	58.4±0.0	0.1±0.0	64.8±0.4	73.7±0.2	83.5±0.8	5.3±4.5
Potato:								
Untreated	ND	ND	ND	ND	58.1±0.1[sd]	62.4±0.1	72.5±0.1	18.9±0.3
in powder form	ND	ND	ND	ND	54.9±0.4	61.6±0.1	72.3±0.4	17.2±0.4
in EtOH (1:1)	ND	ND	ND	ND	57.4±0.3	61.7±0.4	71.9±1.3	16.1±2.1
in H_2O (1:1)	ND	ND	ND	ND	58.7±0.2	63.4±0.3	73.5±0.9	14.4±0.1
in H_2O (1:1), Di	ND	ND	ND	ND	58.3±0.4	63.1±0.4	74.2±1.0	13.5±0.3
in H_2O (2:1)	ND	ND	ND	ND	58.7±0.1	63.8±0.2	73.8±0.5	12.7±0.7
in H_2O (2:1), Di	ND	ND	ND	ND	59.3±0.5	64.4±0.3	74.4±0.8	9.0±1.0
70% amylose maize:								
Untreated	ND	ND	ND	ND	70.6±0.3[sd]	85.7±0.7	113.1±0.9	13.0±1.0
in powder form	ND	ND	ND	ND	66.6±1.3	97.3±0.9	123.5±1.3	11.7±0.9
in EtOH (1:1)	ND	ND	ND	ND	67.5±0.5	92.9±8.2	124.9±1.6	13.4±0.4
in H_2O (1:1)	ND	ND	ND	ND	73.1±2.5	91.6±0.1	124.1±1.2	13.0±0.7
in H_2O (2:1)	ND	ND	ND	ND	71.9±0.7	96.8±0.3	124.5±0.6	12.1±0.9

a,b,c,d are onset, peak,and completion temperature, and enthalpy change, respectively.

sd is standard deviation. ND is not detected. Di is directly after pressure treatment.

Table 7. The Thermal properties of 1 hr HHP treated A-type starches determined by Differential Scanning Calorimetry (trial 1)

	Peak 1				Peak 2			
Starch Type	**To[a] (°C)**	**Tp[b] (°C)**	**Tc[c] (°C)**	**ΔH[d] (J/g)**	**To (°C)**	**Tp (°C)**	**Tc (°C)**	**ΔH (J/g)**
Normal Maize:								
Untreated	ND	ND	ND	ND	66.0±0.1[sd]	70.1±0.2	80.2±0.0	14.0±0.7
in powder form	ND	ND	ND	ND	61.2±0.0	67.2±0.0	79.5±0.4	12.3±0.8
in EtOH (1:1)	ND	ND	ND	ND	65.7±0.1	69.4±0.1	80.4±0.3	13.2±0.1
in H_2O (1:1)	ND	ND	ND	ND	64.0±0.4	69.6±0.6	78.0±0.6	1.7±0.2
in H_2O (1:1), Di	42.8±0.3	51.5±0.8	64.4±2.3	1.6±0.2	68.6±1.3	75.1±2.0	81.7±2.4	0.5±0.2
in H_2O (2:1)	41.5±0.9	49.7±3.0	64.0±1.6	1.9±0.4	ND	ND	ND	ND
in H_2O (2:1), Di	43.3±0.4	48.7±2.3	58.6±0.6	0.2±0.1	ND	ND	ND	ND
Waxy Maize:								
Untreated	ND	ND	ND	ND	64.6±0.2[sd]	70.4±0.3	81.2±0.4	16.1±0.2
in powder form	ND	ND	ND	ND	62.3±0.2	68.7±0.2	80.5±1.0	13.9±0.2
in EtOH (1:1)	ND	ND	ND	ND	64.0±0.5	69.9±0.2	83.0±1.5	15.4±0.3
in H_2O (1:1)	42.4±1.4	47.7±1.3	58.0±0.3	0.5±0.2	64.9±0.4	71.2±0.2	81.0±0.6	3.8±0.3
in H_2O (1:1), Di	44.1±0.4	54.2±0.9	66.1±0.1	3.2±0.4	69.3±0.5	76.1±0.5	87.7±0.2	2.5±0.3
in H_2O (2:1)	39.8±0.7	51.3±1.5	71.6±0.4	6.0±0.3	ND	ND	ND	ND
in H_2O (2:1), Di	41.3±1.0	56.3±0.3	65.9±0.1	0.9±0.3	ND	ND	ND	ND
Tapioca:								
Untreated	ND	ND	ND	ND	64.9±0.1[sd]	69.1±0.1	82.2±0.5	14.8±0.3
1hr,Dry Pwd	ND	ND	ND	ND	60.3±0.1	66.6±0.1	79.0±0.0	12.2±0.3
1hr,in EtOH	ND	ND	ND	ND	64.8±0.0	68.9±0.1	81.8±0.6	14.6±0.2
1hr,1:1 H_2O	43.3±0.3	51.9±0.8	60.2±2.0	0.3±0.1	65.5±0.2	70.6±0.5	78.2±0.4	0.6±0.2
1hr,1:1 H_2O, Di	41.9±0.2	48.9±2.3	58.0±1.1	0.4±0.0	64.0±1.0	71.1±0.9	80.1±0.2	2.0±0.2
1hr,2:1 H_2O	45.1±0.4	48.1±2.1	56.2±0.7	0.3±0.1	ND	ND	ND	ND
1hr,2:1 H_2O, Di	ND	ND	ND	ND	ND	ND	ND	ND

a,b,c,d are onset, peak,and completion temperature, and enthalpy change, respectively.

sd is standard deviation. ND is not detected. Di is directly after pressure treatment.

Table 7. (continued)

Starch Type	Peak 1				Peak 2			
	To[a] (°C)	Tp[b] (°C)	Tc[c] (°C)	ΔH[d] (J/g)	To (°C)	Tp (°C)	Tc (°C)	ΔH (J/g)
Rice:								
Untreated	ND	ND	ND	ND	60.6±0.6[sd]	68.3±0.1	84.7±2.3	16.8±4.1
in powder form	ND	ND	ND	ND	56.0±0.6	66.0±0.4	83.0±0.6	14.2±0.3
in EtOH (1:1)	ND	ND	ND	ND	59.8±0.1	67.7±0.4	86.3±3.6	17.7±1.9
in H_2O (1:1)	45.1±0.0	51.0±0.0	55.6±0.0	0.1±0.0	64.7±0.8	72.3±1.3	80.6±1.3	1.0±0.1
in H_2O (1:1), Di	45.3±0.4	50.9±0.4	58.4±0.6	0.2±0.0	65.7±0.6	75.0±0.9	85.0±1.4	2.8±0.1
in H_2O (2:1)	ND	ND	ND	ND	ND	ND	ND	ND
in H_2O (2:1), Di	ND	ND	ND	ND	ND	ND	ND	ND
Potato:								
Untreated	ND	ND	ND	ND	58.1±0.1[sd]	62.4±0.1	72.5±0.1	18.9±0.3
in powder form	ND	ND	ND	ND	54.4±0.3	61.0±0.2	71.3±0.6	18.4±0.3
in EtOH (1:1)	ND	ND	ND	ND	56.9±0.1	61.2±0.2	70.9±0.3	18.0±0.3
in H_2O (1:1)	ND	ND	ND	ND	58.7±0.2	62.6±0.1	71.4±0.3	14.8±0.6
in H_2O (1:1), Di	ND	ND	ND	ND	60.1±0.3	64.4±0.3	74.6±0.9	10.8±0.2
in H_2O (2:1)	ND	ND	ND	ND	59.0±0.2	63.8±0.1	72.2±0.1	10.4±0.7
in H_2O (2:1), Di	ND	ND	ND	ND	60.3±0.5	65.8±0.6	75.5±0.8	16.6±9.8
70% amylose maize:								
Untreated	ND	ND	ND	ND	70.6±0.3[sd]	85.7±0.7	113.1±0.9	13.0±1.0
in powder form	ND	ND	ND	ND	68.9±0.5	96.1±5.1	113.4±0.8	11.9±1.6
in EtOH (1:1)	ND	ND	ND	ND	70.0±0.2	91.2±0.3	119.0±6.2	14.5±1.7
in H_2O (1:1)	ND	ND	ND	ND	72.9±0.3	85.7±0.0	111.3±0.4	9.4±0.5
in H_2O (2:1)	ND	ND	ND	ND	74.6±0.5	95.2±6.3	113.0±1.1	10.7±0.4

a,b,c,d are onset, peak,and completion temperature, and enthalpy change, respectively.

sd is standard deviation. ND is not detected. Di is directly after pressure treatment.

Table 8. The Thermal properties of 1 hr HHP treated A-type starches determined by Differential Scanning Calorimetry (trial 2)

	Peak 1				Peak 2			
Starch Type	**To[a] (°C)**	**Tp[b] (°C)**	**Tc[c] (°C)**	**ΔH[d] (J/g)**	**To (°C)**	**Tp (°C)**	**Tc (°C)**	**ΔH (J/g)**
Normal Maize:								
Untreated	ND	ND	ND	ND	66.0±0.1[sd]	70.1±0.2	80.2±0.0	14.0±0.7
in powder form	ND	ND	ND	ND	61.2±0.3	67.3±0.2	77.7±0.5	11.3±1.1
in EtOH (1:1)	ND	ND	ND	ND	65.9±0.7	69.6±0.3	78.8±0.4	13.5±0.6
in H_2O (1:1)	44.4±0.7	51.2±0.5	62.2±0.1	1.7±0.0	66.6±0.3	72.1±0.3	79.4±0.7	0.6±0.1
in H_2O (1:1), Di	44.7±0.6	51.3±0.2	60.6±2.0	0.7±0.1	65.7±0.4	71.9±0.6	79.5±0.4	1.5±0.1
in H_2O (2:1)	42.2±0.6	51.2±0.6	63.2±0.7	0.3±0.1	ND	ND	ND	ND
in H_2O (2:1), Di	ND	ND	ND	ND	ND	ND	ND	ND
Waxy Maize:								
Untreated	ND	ND	ND	ND	64.6±0.2[sd]	70.4±0.3	81.2±0.4	16.1±0.2
in powder form	ND	ND	ND	ND	61.9±0.2	68.4±0.1	81.7±1.7	14.9±0.5
in EtOH (1:1)	ND	ND	ND	ND	64.2±0.2	69.9±0.1	80.9±0.2	15.9±0.2
in H_2O (1:1)	45.0±1.8	52.4±0.1	63.2±0.7	2.7±0.2	66.6±0.2	72.1±0.3	80.1±1.0	2.0±0.1
in H_2O (1:1), Di	44.2±3.3	51.9±0.6	60.7±2.8	0.6±0.3	66.8±1.0	73.6±0.8	84.1±1.3	4.3±0.8
in H_2O (2:1)	42.5±0.6	49.9±0.5	70.7±0.3	5.5±0.3	ND	ND	ND	ND
in H_2O (2:1), Di	42.4+0.0	58.3+0.0	66.7+0.0	0.2+0.0	ND	ND	ND	ND
Tapioca:								
Untreated	ND	ND	ND	ND	64.9±0.1[sd]	69.1±0.1	82.2±0.5	14.8±0.3
1hr,Dry Pwd	ND	ND	ND	ND	57.1±0.3	63.7±0.2	75.4±0.5	12.7±0.1
1hr,in EtOH	ND	ND	ND	ND	61.9±0.2	66.2±0.3	79.7±0.2	15.2±0.1
1hr,1:1 H_2O	44.8±0.0	50.5±0.0	54.1±0.0	0.2±0.0	62.1±0.2	68.3±0.1	75.4±0.4	0.9±0.0
1hr,1:1 H_2O, Di	46.7±0.0	52.7±0.0	59.1±0.0	0.2±0.0	65.4±0.4	70.7±1.3	78.7±0.3	1.4±0.1
1hr,2:1 H_2O	42.8±0.6	48.5±1.9	55.2±2.1	0.6±0.2	ND	ND	ND	ND
1hr,2:1 H_2O, Di	ND	ND	ND	ND	ND	ND	ND	ND

a,b,c,d are onset, peak,and completion temperature, and enthalpy change, respectively.

sd is standard deviation. ND is not detected. Di is directly after pressure treatment.

Table 8. (continued)

Starch Type	Peak 1				Peak 2			
	To[a] (°C)	Tp[b] (°C)	Tc[c] (°C)	ΔH[d] (J/g)	To (°C)	Tp (°C)	Tc (°C)	ΔH (J/g)
Rice:								
Untreated	ND	ND	ND	ND	60.6±0.6[sd]	68.3±0.1	84.7±2.3	16.8±4.1
in powder form	ND	ND	ND	ND	56.4±0.4	66.2±0.2	81.5±1.1	11.1±0.8
in EtOH (1:1)	ND	ND	ND	ND	60.3±0.1	68.0±0.0	83.5±0.3	13.9±0.5
in H_2O (1:1)	47.2±1.6	52.1±1.7	57.0±0.4	0.2±0.1	65.0±0.2	73.3±0.3	83.1±0.7	2.1±0.2
in H_2O (1:1), Di	ND	ND	ND	ND	66.1±0.0	77.7±0.0	83.9±0.0	0.6±0.0
in H_2O (2:1)	44.4±0.3	51.2±0.1	58.8±1.7	0.2±0.0	65.6±0.8	74.1±1.1	81.0±1.1	1.1±0.3
in H_2O (2:1), Di	46.4±0.0	51.5±0.0	59.4±0.0	0.1±0.0	66.5±0.7	75.4±1.1	83.9±1.0	2.6±1.1
Potato:								
Untreated	ND	ND	ND	ND	58.1±0.1[sd]	62.4±0.1	72.5±0.1	18.9±0.3
in powder form	ND	ND	ND	ND	54.6±0.3	61.4±0.1	71.8±0.5	17.1±0.4
in EtOH (1:1)	ND	ND	ND	ND	57.0±0.3	61.2±0.4	70.9±0.9	16.9±0.6
in H_2O (1:1)	ND	ND	ND	ND	59.8±0.3	64.2±0.3	73.8±0.4	14.9±0.1
in H_2O (1:1), Di	ND	ND	ND	ND	60.3±0.0	64.5±0.0	74.3±0.3	13.1±0.2
in H_2O (2:1)	ND	ND	ND	ND	60.1±0.2	64.8±0.3	74.4±0.1	12.1±0.3
in H_2O (2:1), Di	ND	ND	ND	ND	60.3±1.1	65.6±0.7	74.7±0.4	17.7±13.6
70% amylose maize:								
Untreated	ND	ND	ND	ND	70.6±0.3[sd]	85.7±0.7	113.1±0.9	13.0±1.0
in powder form	ND	ND	ND	ND	66.2±0.2	97.2±1.7	123.1±1.8	11.5±1.1
in EtOH (1:1)	ND	ND	ND	ND	67.0±0.6	86.7±8.5	123.7±1.0	14.4±0.4
in H_2O (1:1)	ND	ND	ND	ND	70.3±0.4	90.3±0.3	123.4±0.8	14.5±0.1
in H_2O (2:1)	ND	ND	ND	ND	71.5±0.0	93.9±3.7	122.4±0.5	13.3±1.9

a,b,c,d are onset, peak,and completion temperature, and enthalpy change, respectively.

sd is standard deviation. ND is not detected. Di is directly after pressure treatment.

APPENDIX A. ADDITIONAL TABLES

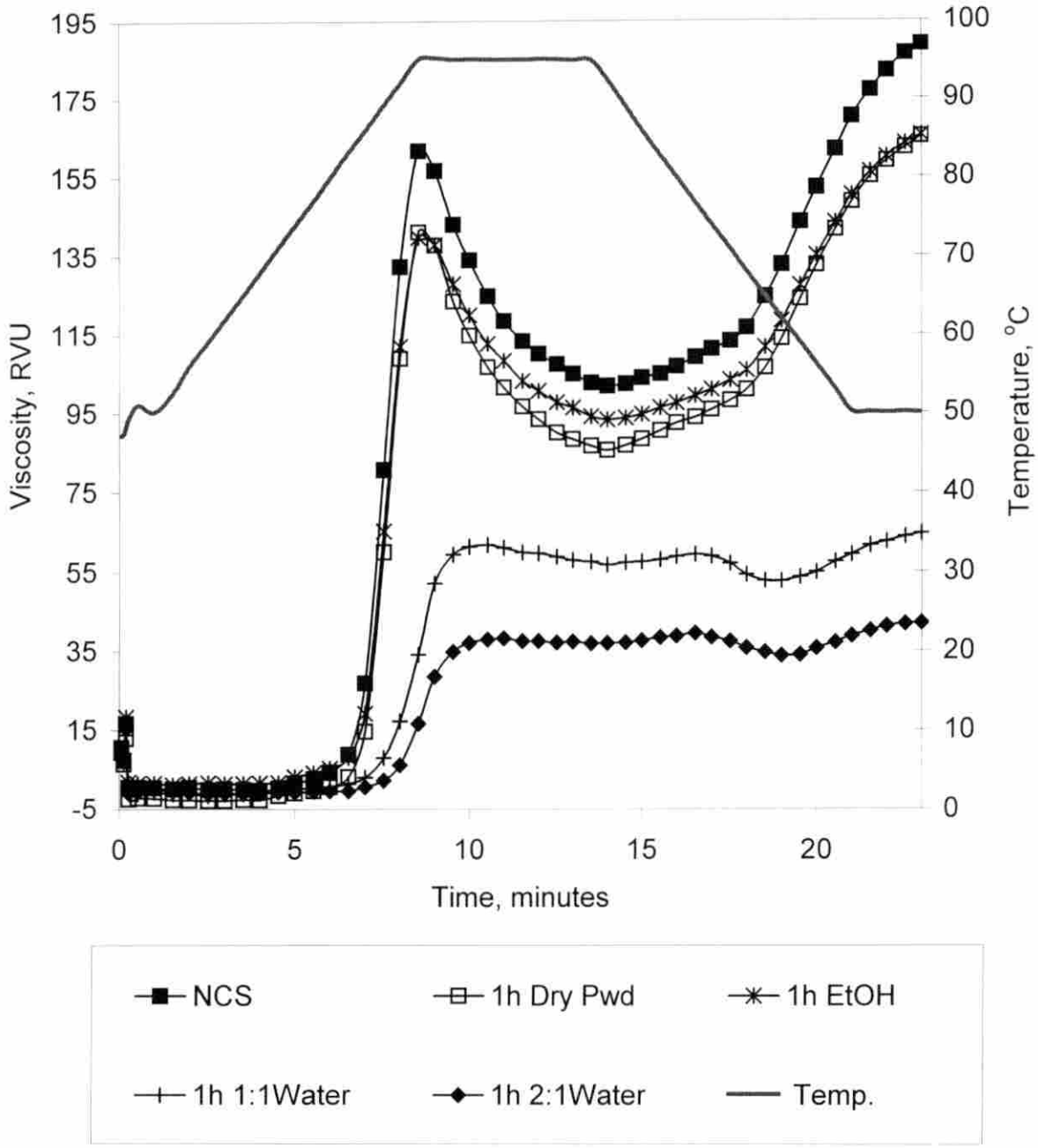

Figure 1. The RVA curve for normal maize starch with 8% solid concentration and 160 rpm spindle speed (1hr press).

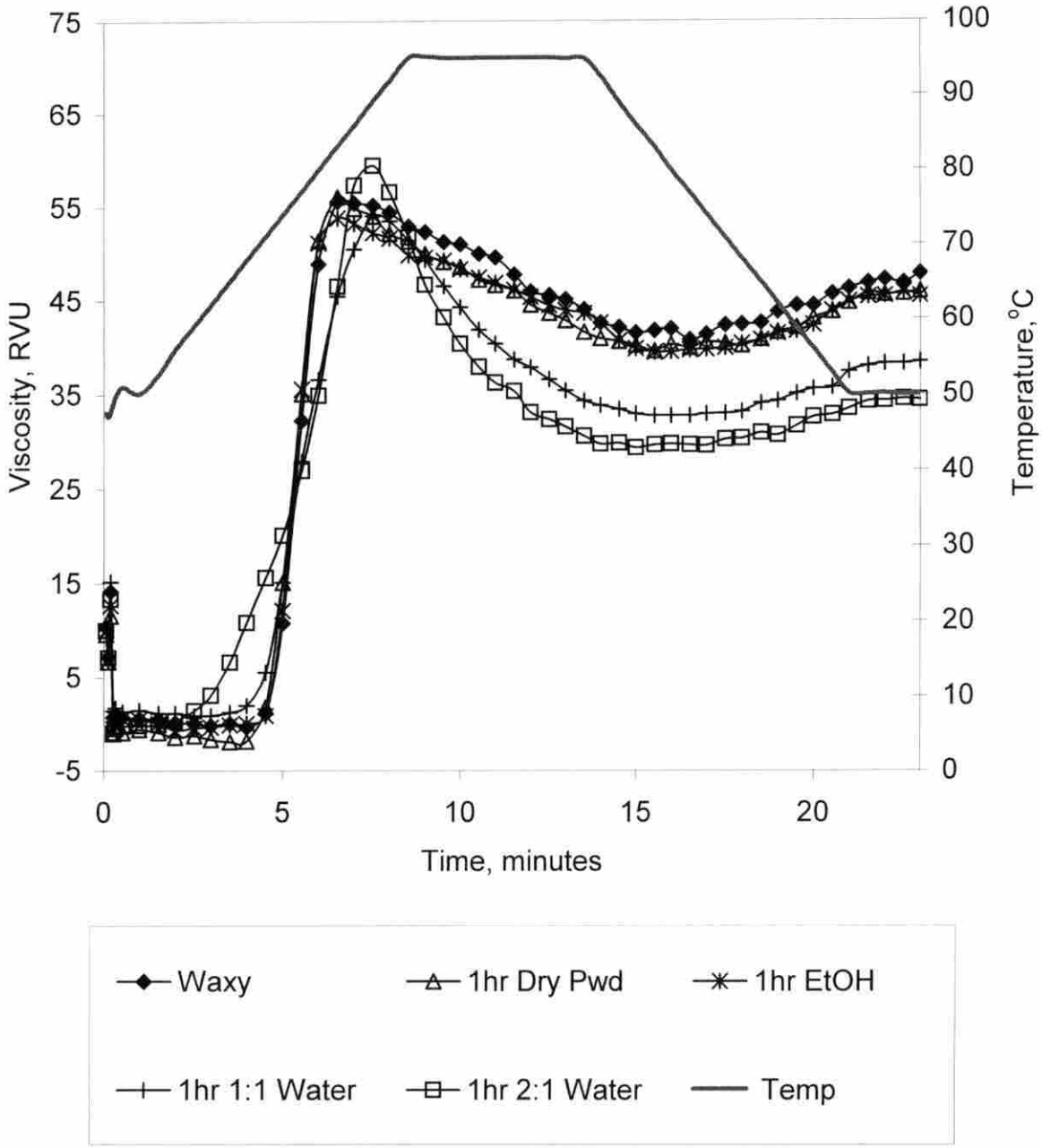

Figure 2. The RVA curve for waxy maize starch with 4% solid concentration and 160 rpm spindle speed (1hr press).

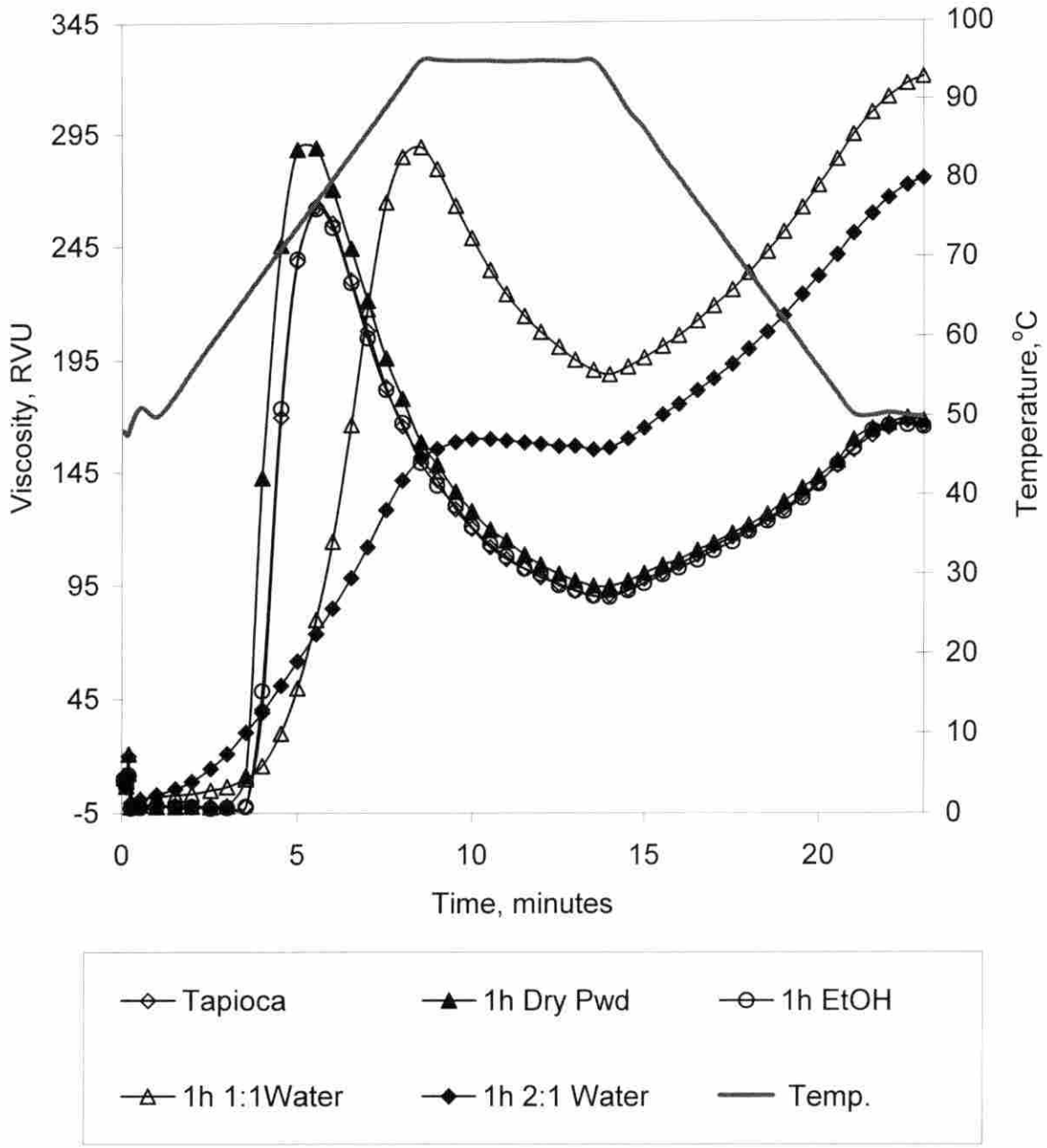

Figure 3. The RVA curve for tapioca starch with 8% solid concentration and 160 rpm spindle speed (1hr press).

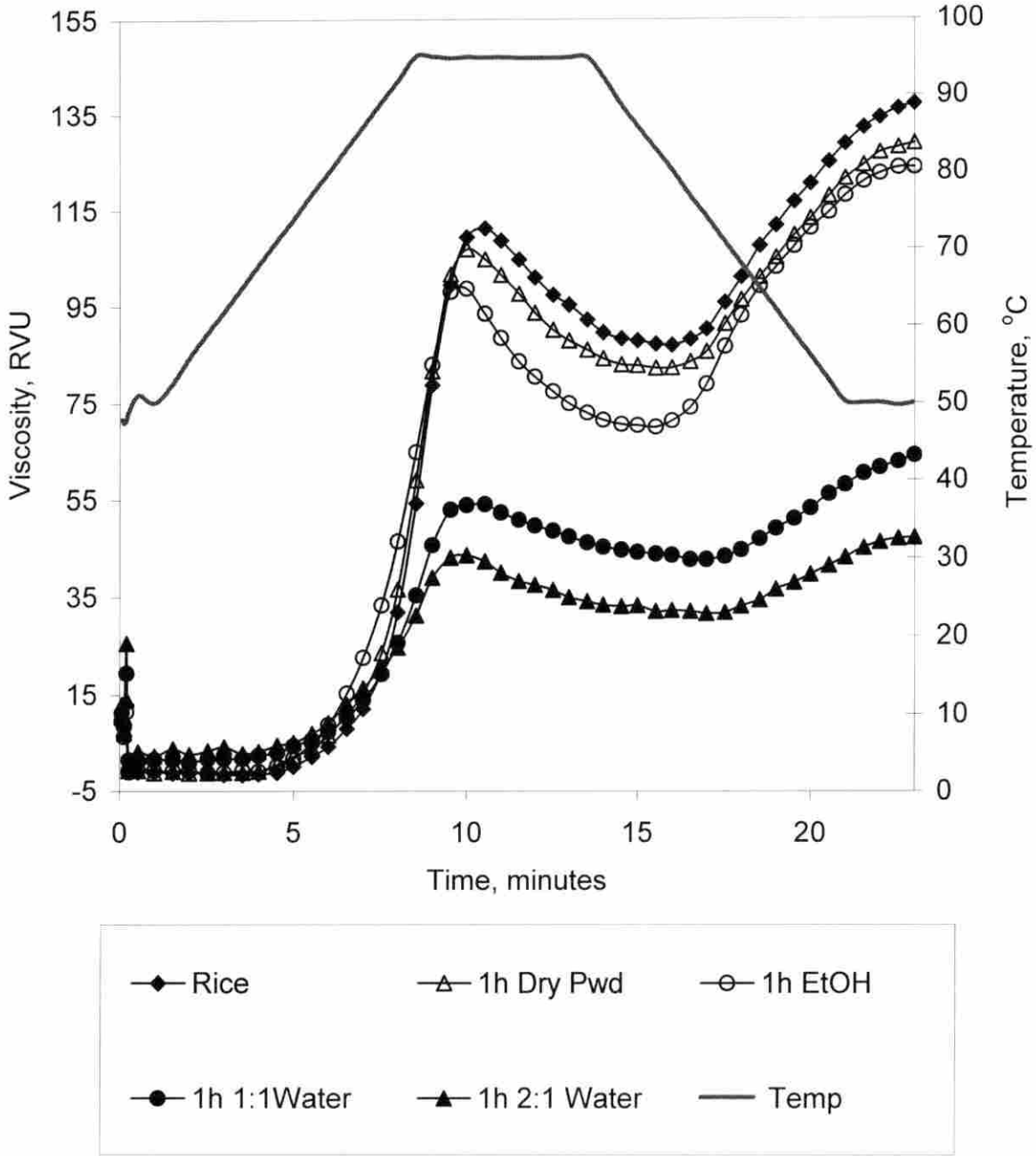

Figure 4. The RVA curve for rice starch with 8% solid concentration and 160 rpm spindle speed (1hr press).

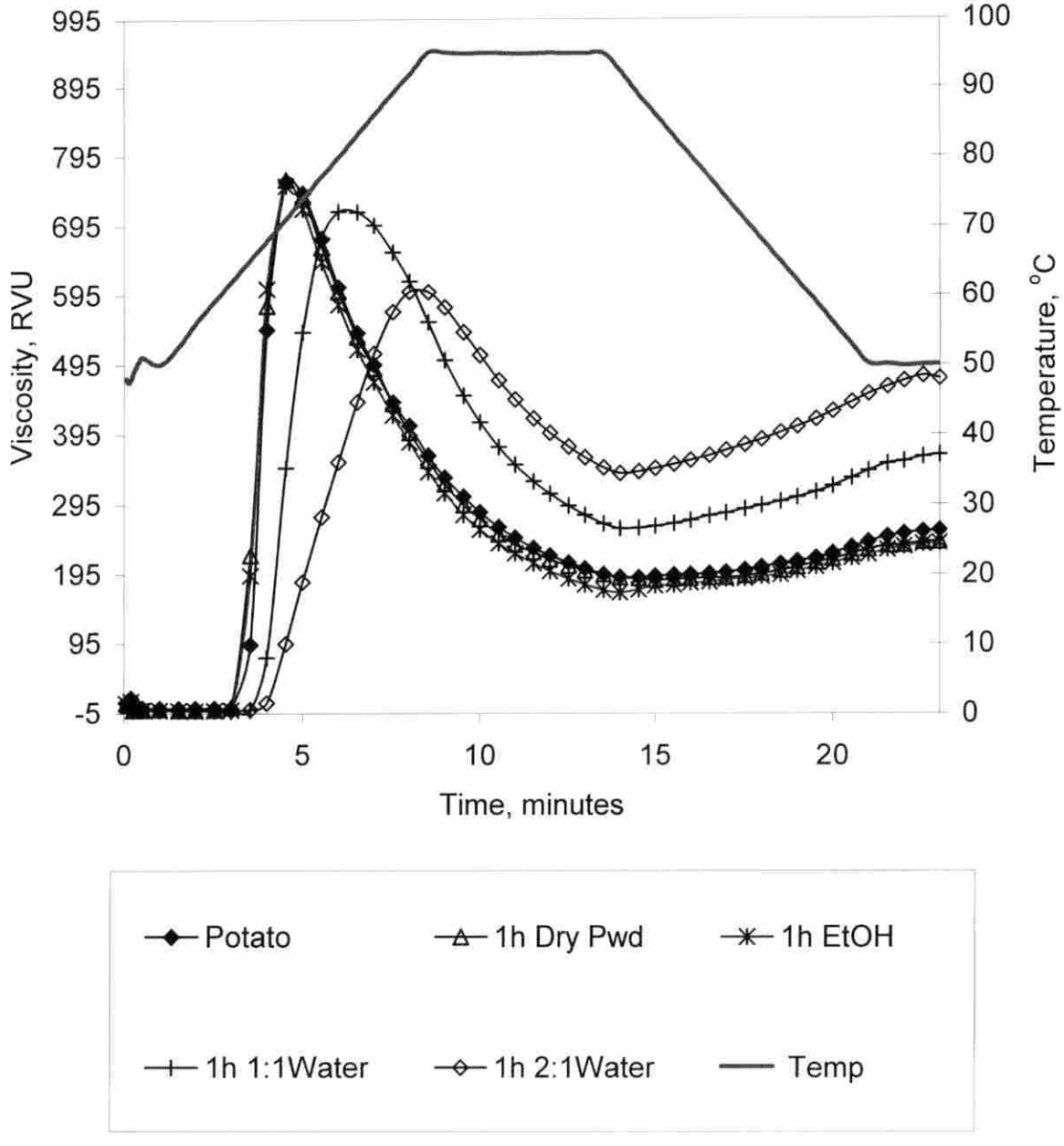

Figure 5. The RVA curve for potato starch with 8% solid concentration and 160 rpm spindle speed (1hr press).

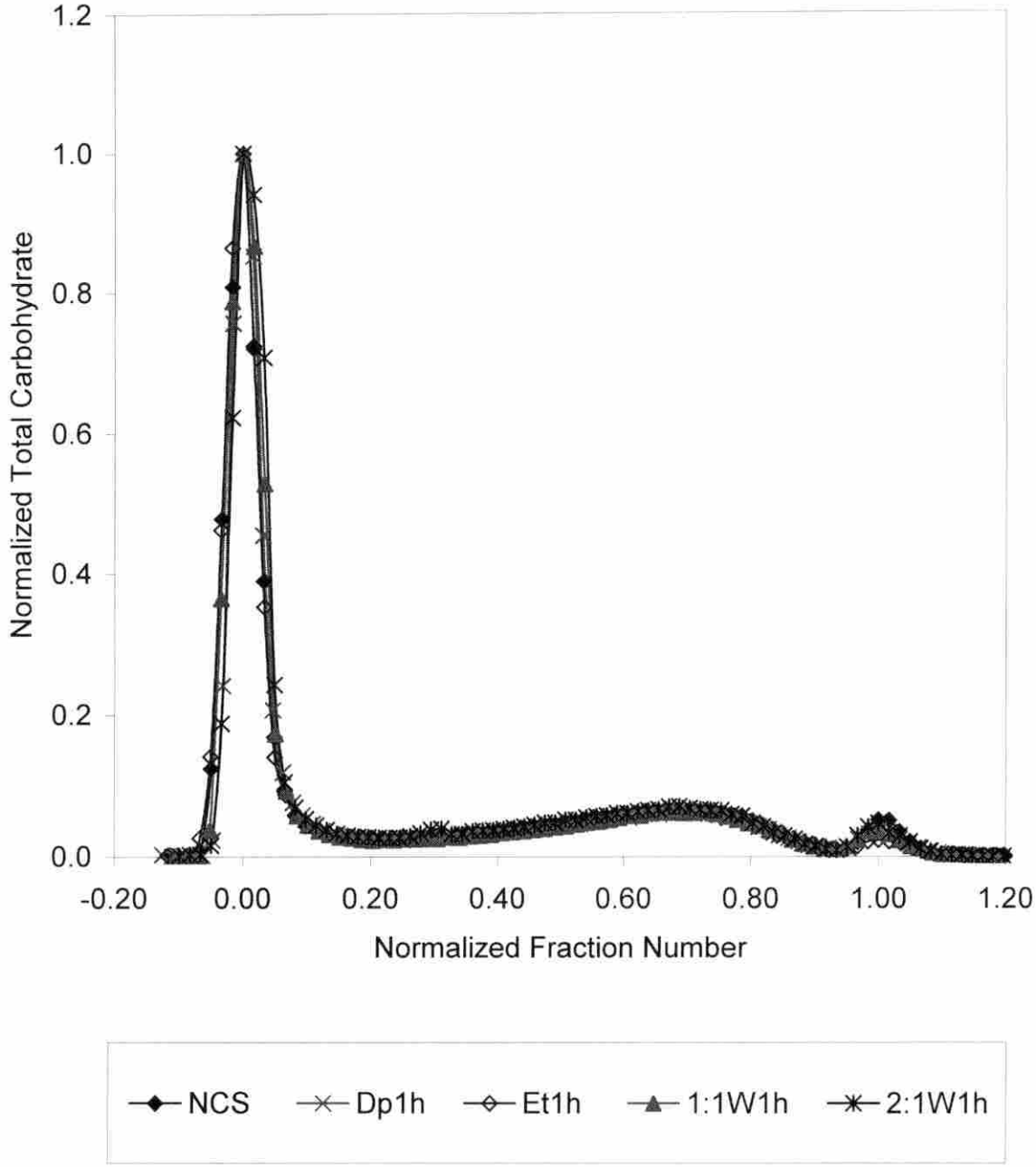

Figure 6a. The total CHO curve from 15 mg of normal maize starch separated in Sepharose CL-2B (1 hour dwell time)

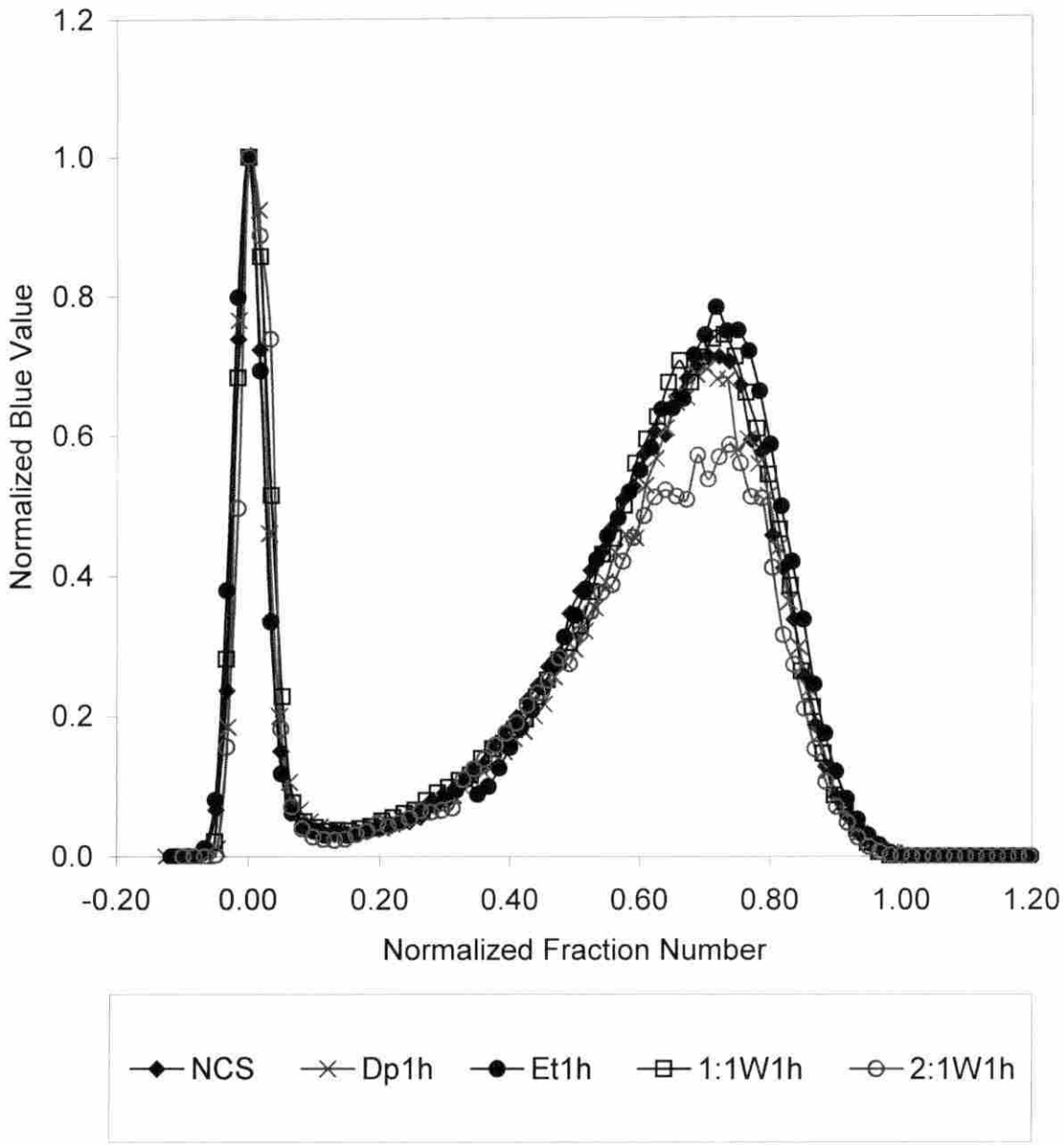

Figure 6b. The blue value curve from 15 mg of normal maize starch separated in Sepharose CL-2B (1 hour dwell time)

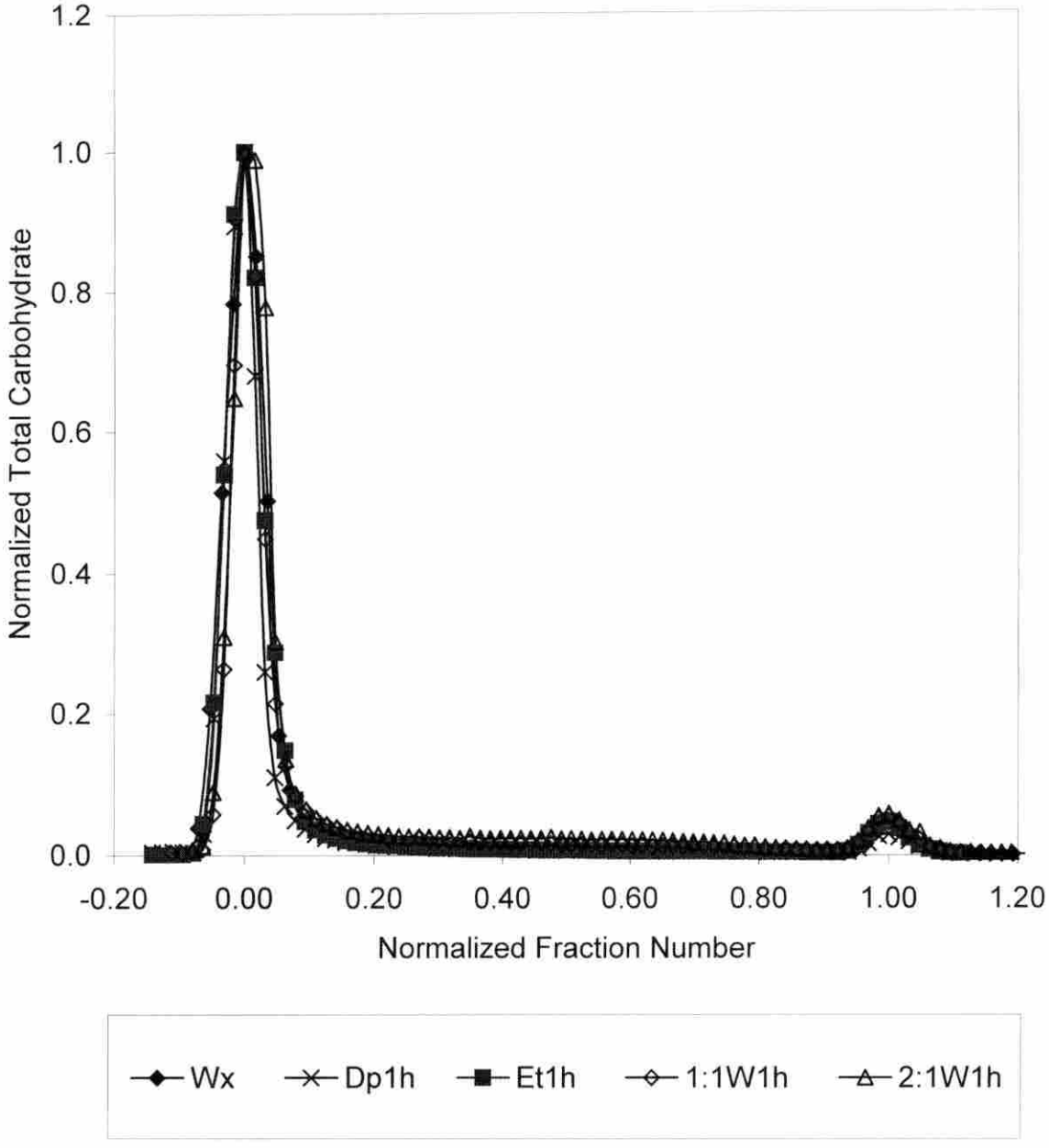

Figure 7a. The total CHO curve from 15 mg of waxy maize starch separated in Sepharose CL-2B (1 hour dwell time)

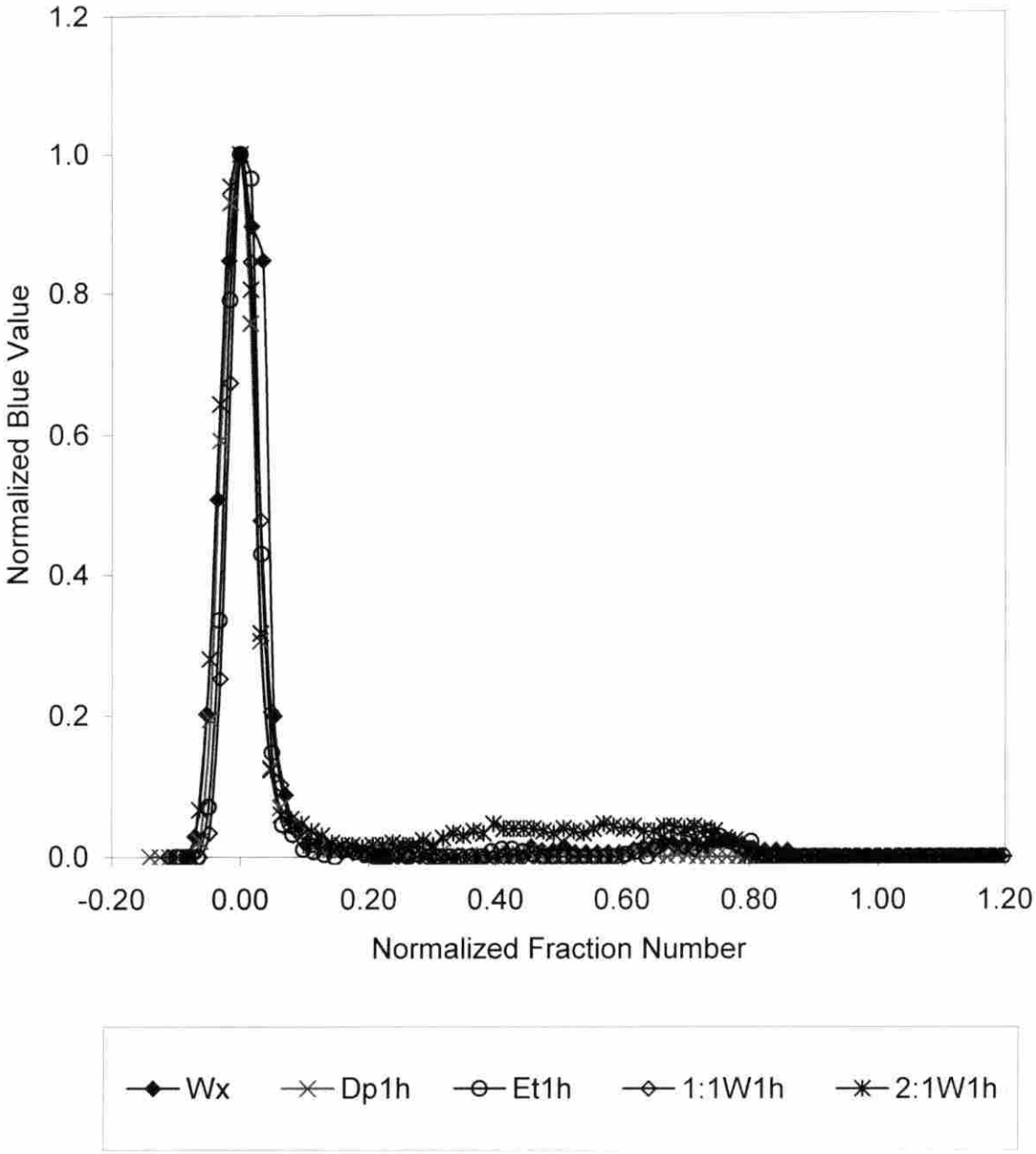

Figure 7b. The blue value curve from 15 mg of waxy maize starch separated in Sepharose CL-2B (1 hour dwell time)

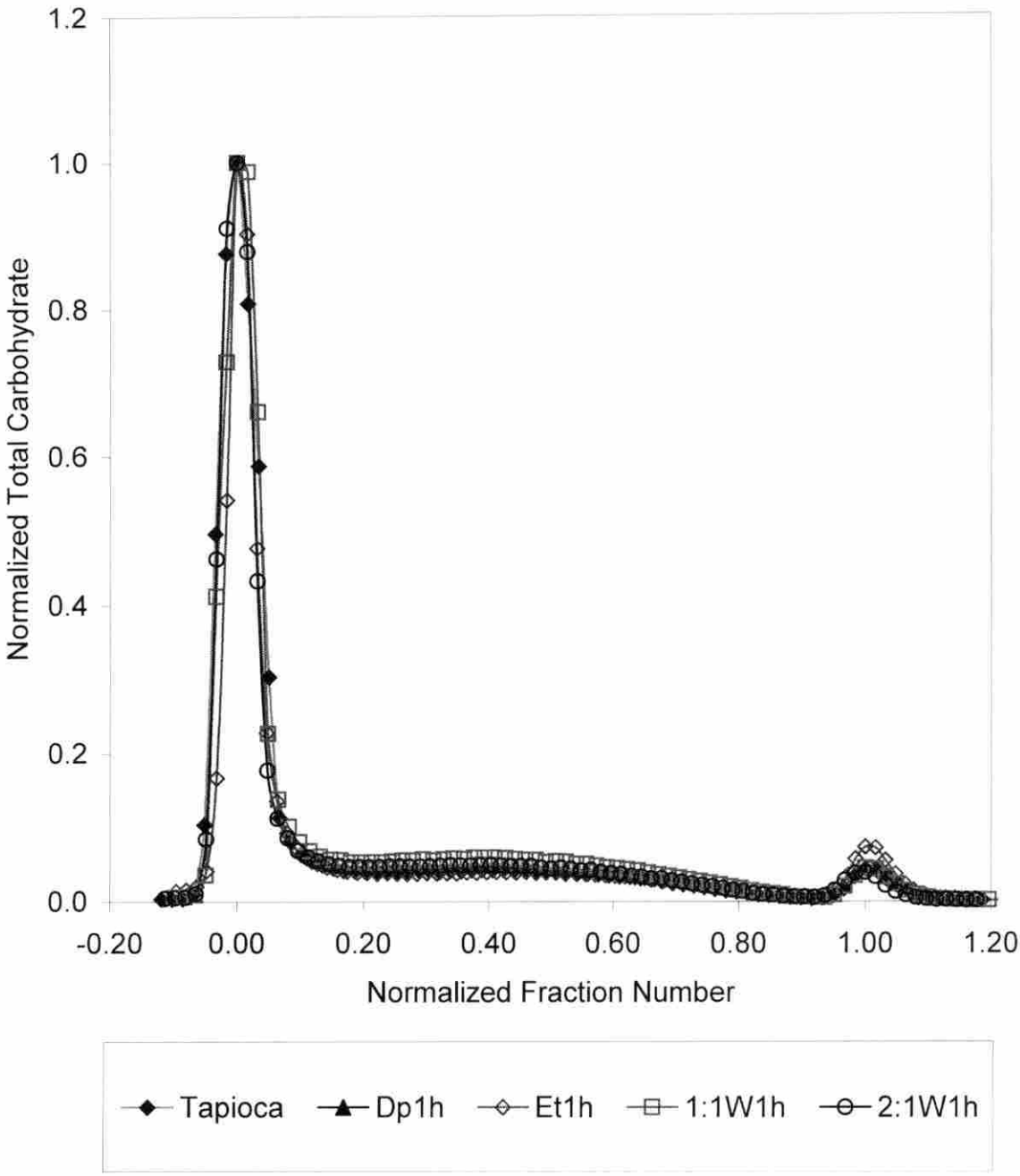

Figure 8a. The total CHO curve from 15 mg of tapioca starch separated in Sepharose CL-2B (1 hour dwell time)

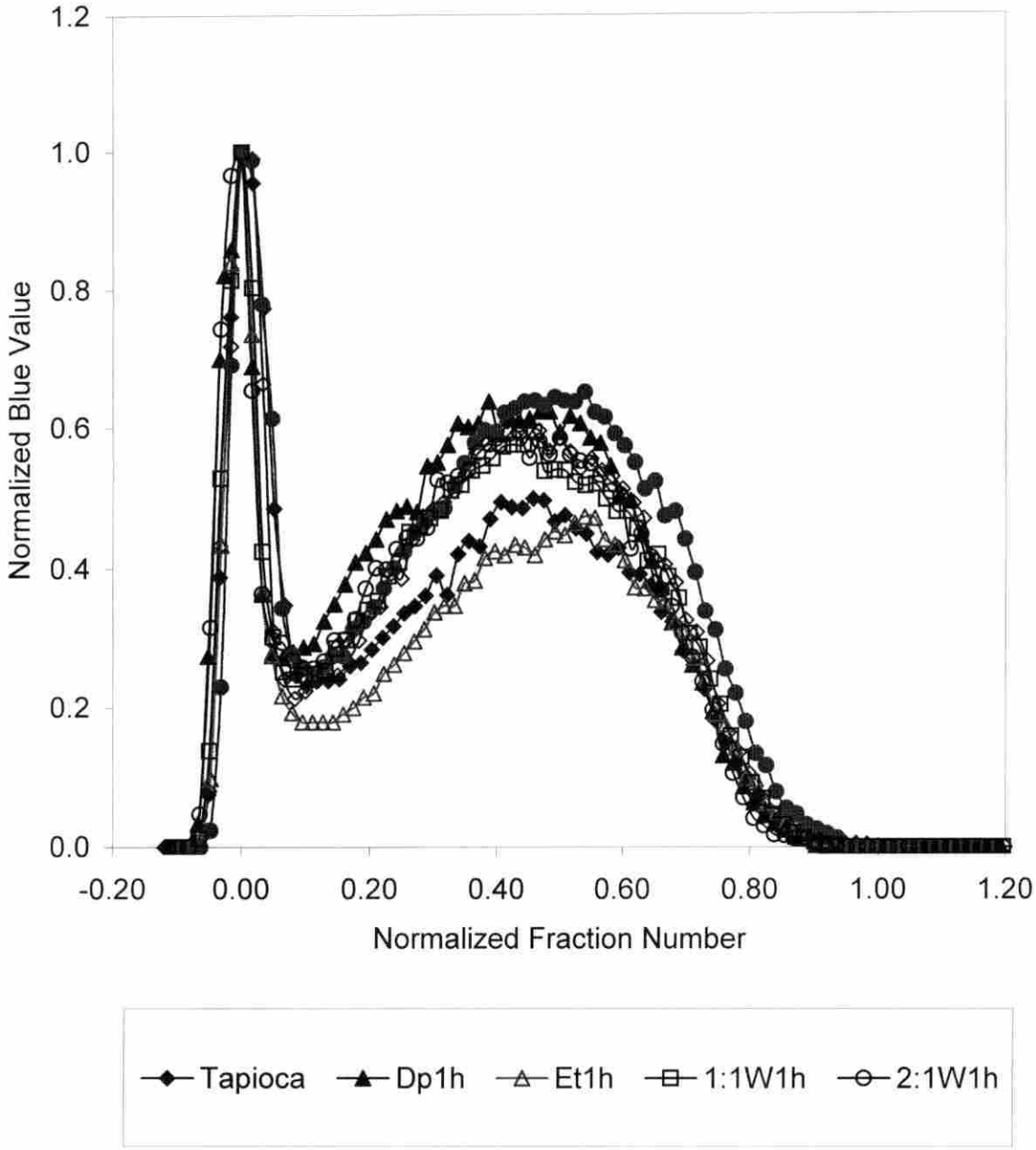

Figure 8b. The blue value curve from 15 mg of tapioca starch separated in Sepharose CL-2B (1 hour dwell time)

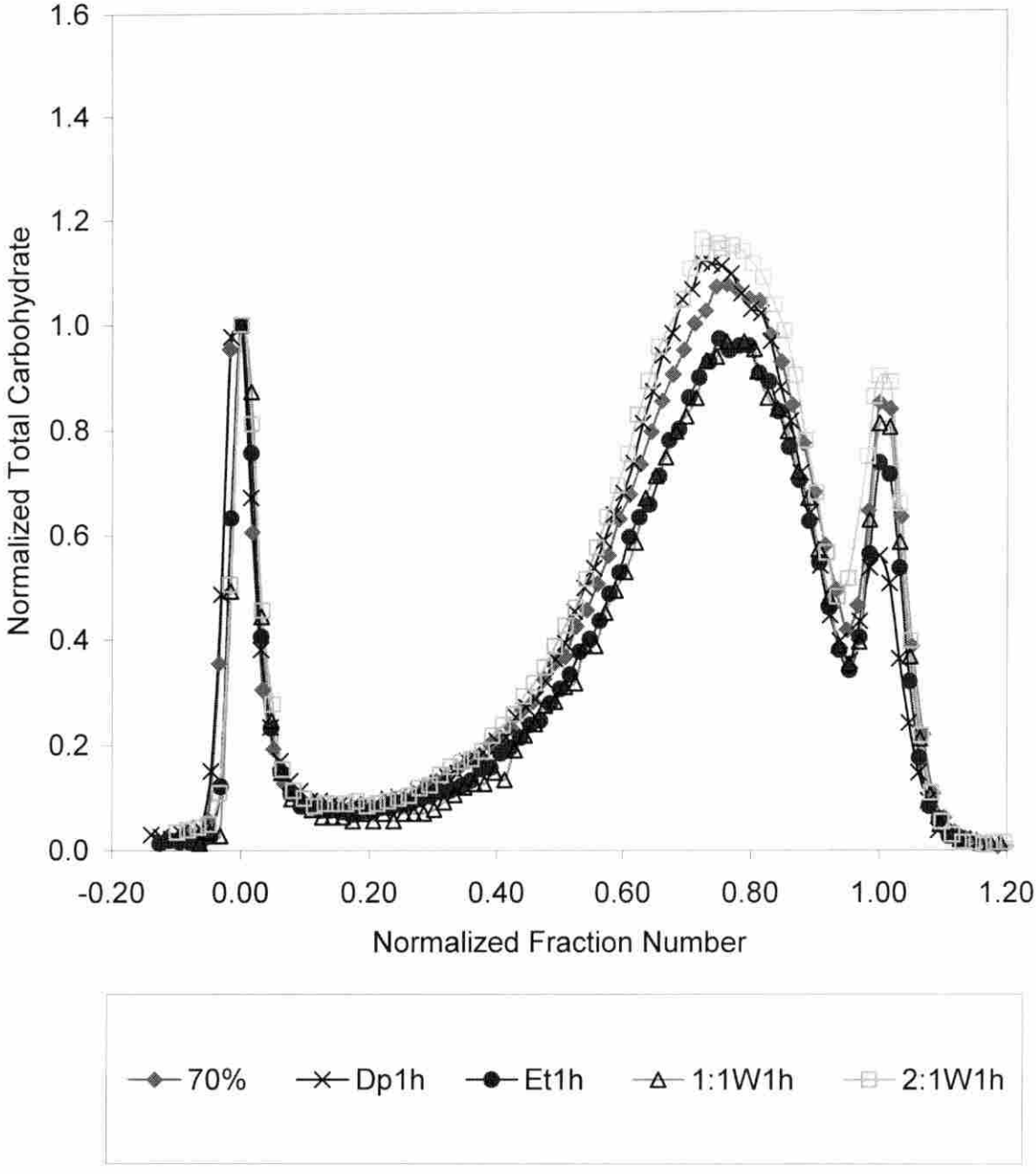

Figure 9a. The total CHO curve from 15 mg of high amylose maize starch separated in Sepharose CL-2B (1 hour dwell time)

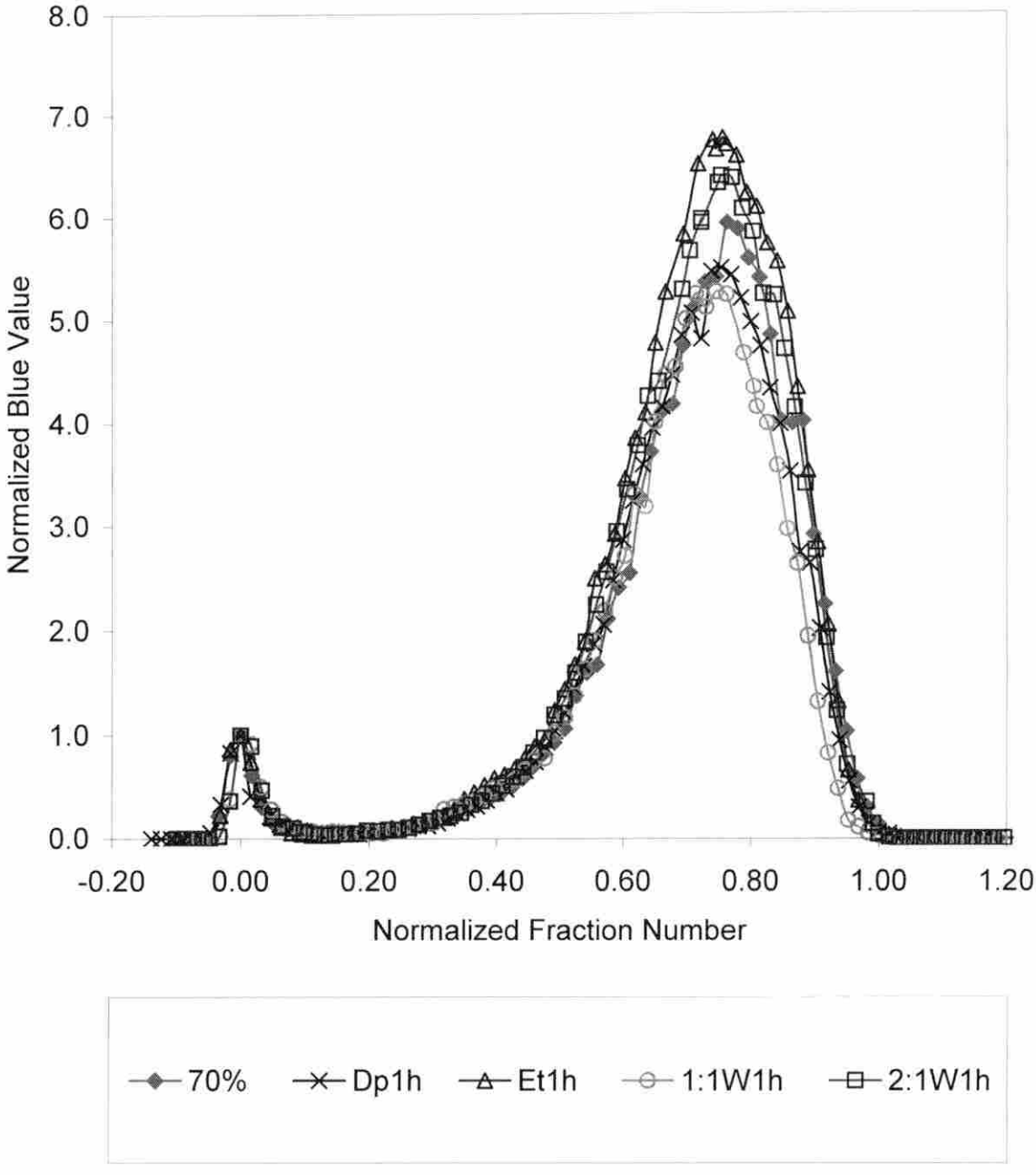

Figure 9b. The blue value curve from 15 mg of high amylose maize starch separated in Sepharose CL-2B (1 hour dwell time)

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